

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 January 2004 (08.01.2004)

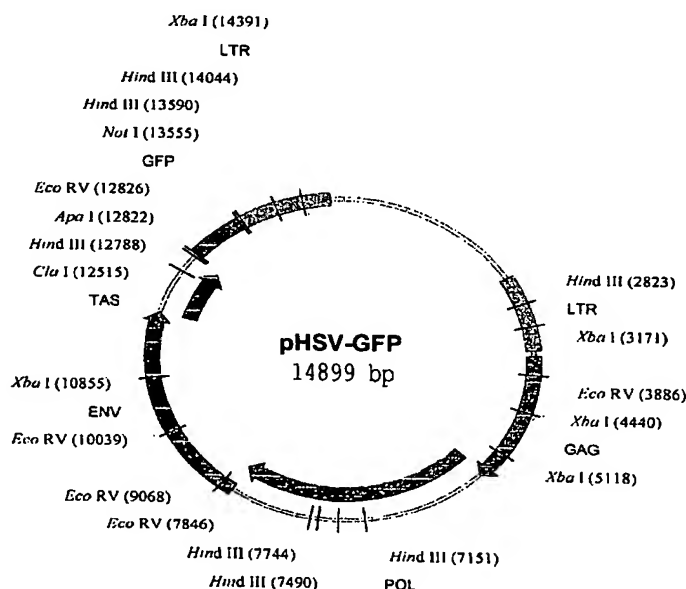
PCT

(10) International Publication Number
WO 2004/003153 A2

- (51) International Patent Classification⁷: **C12N**
- (21) International Application Number: **PCT/US2003/020325**
- (22) International Filing Date: **27 June 2003 (27.06.2003)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/392,630 27 June 2002 (27.06.2002) US
- (71) Applicant (for all designated States except US): **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as REPRESENTED BY THE SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES CENTERS FOR DISEASE CONTROL AND PREVENTION [US/US]; Technology Transfer Office 4770 Buford Highway, (K-79), Atlanta, GA 30341 (US).**
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **FOLKS, Thomas, M. [US/US]; 3815 Belle Glade Trail, Snellville, GA 30039**
- (74) Agents: **NEEDLE & ROSENBERG, P.C. et al.; Suite 1000, 999 Peachtree Street, Atlanta, GA 30309-3915 (US).**
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.**
- (84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).**
- Published:
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: **LIVE REPLICATING SPUMAVIRUS VECTOR**



(57) Abstract: The present invention provides a vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid. The present invention also provides methods of treating or preventing a condition resulting from a viral, bacterial, or parasitic infection in a subject comprising administering to the subject an effective amount of the vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid. Also provided in the present invention are methods of treating a condition resulting from a cancer in a subject comprising administering to the subject an effective amount of the vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid.

WO 2004/003153 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

LIVE REPLICATING SPUMAVIRUS VECTOR

BACKGROUND OF THE INVENTION

- 5 1. Spumavirus, also known as foamy virus for the characteristics of vacuolization the virus induces in cell culture, belongs to a distinct group of retroviruses. The simian foamy viruses (SFVs) include isolates from Old World and New World monkeys and are classified into 10 different serotypes based on serological cross-reactivities. Virus appears to persist in the host for a long period of time in a latent form and can exist in the presence of neutralizing antibody.
- 10 2. Currently the most studied retrovirus, Human Immunodeficiency Virus, is believed to be derived from nonhuman primate transmission into humans at some past time. Concerns about the risk of transmission of retroviruses from non-human primates to humans working in research laboratories were heightened in the early 1990's when two persons developed antibodies to SIV (Simian Immunodeficiency Virus) following work-related exposures, one of whom had clear evidence of persistent viral infection. (See CDC anonymous survey for simian immunodeficiency virus (SIV) seropositivity in SIV laboratory researchers – United States, 1992. MMWR Morb. Mort. Wkly. Rep. 1992; 41:814-5; Khabbaz R.F., et al. Brief report: infection of a laboratory worker with simian immunodeficiency virus. New Eng. J. Med. 1994; 330:172-7; Khabbaz R.F., et al. Simian immunodeficiency virus needle stick accident in a laboratory worker. Lancet 1992; 340:271-3; and CDC. Guideline to prevent simian immunodeficiency virus infection in laboratory workers and animal handlers. MMWR 1988; 37:693-704.) In addition to SIV, nonhuman primate species used in biomedical research are commonly infected with SFV (simian foamy virus), STLV (simian t-cell lymphotropic virus), and/or type D retroviruses. All of these retroviruses cause lifelong infections in nonhuman primates, and some are known to be transmissible through sexual contact, blood, or breast-feeding. Natural SFV infections in non-human primates have not been definitively associated with disease. In non-human primates, infection with the other retroviruses may result in a clinical spectrum ranging from asymptomatic infection to life threatening immunodeficiency syndromes or lymphoproliferative disorders. The transmission routes of SFVs among non-human primates remain undefined, but the prevalence of seroreactivity is high among captive adult non-human primates.

3. Recent publications indicate that earlier serological tests showing human spumavirus antibodies in the human populating were incorrect. Immunological investigation of a previously reported human spumavirus revealed that it shared common antigens in complement fixation, immunofluorescence and neutralization assays with the chimpanzee foamy virus, SFV-6. Furthermore, failure to detect serological evidence of HFV infection in people from a wide geographical area suggested that this virus isolate was a variant of SFV-6 particularly since sera from chimpanzees naturally infected with SFV-6 neutralized both viruses. In a survey for prevalence of human foamy virus in more than 5000 human sera, collected from geographically diverse populations, none of the serum samples were confirmed as positive. Taken together with sequence analysis endorsing the phylogenetic closeness of the purported human spumavirus to SFV-6/7, these data strongly suggest that human foamy virus is not naturally found in the human populations. (See Ali, M. et al., "No Evidence of Antibody to Human Foamy Virus in Widespread Human Populations," AIDS Research and Human Retroviruses, Vol. 12, NO. 15, 1996).

4. Gene therapies have long looked for a good vector that can transport the foreign gene of choice into human cells. Thus, compositions and methods for gene therapy are needed that use a vector capable of carrying a significant amount of foreign DNA that will enter the host organism and not cause disease.

5. Compositions and methods for vaccination using recombinant live retroviruses are also needed. A live virus, that causes no illness in humans, and that has genes of antigens of choice incorporated into its genome, would provide for an excellent vaccination tool as a vector. The retrovirus would reproduce in the human host and expose the immune system to antigens so that an immune response can be initiated.

6. Targeted attack on reproducing cells is a goal of cancer treatment. What is needed are compositions and methods for cancer treatment that are specific for dividing cells that do not cause systemic damage to the cancer patient. A viral vector that could infect and kill dividing cells, without killing other cells of the host would provide a solution for cancer treatment.

7. The lack of any known disease associated with the virus from which the vector of the present invention was derived makes the present invention ideal for gene therapy regimens.

SUMMARY OF THE INVENTION

5 8. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to live replicating retroviral vectors and methods of their use.

9. Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be
10 learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

15 10. The present invention provides compositions comprising live replicating retroviral vectors, wherein the vector is derived from a spumavirus, and wherein the vector further comprises a nucleic acid that encodes a non-spumavirus peptide, polypeptide, or protein. Thus, the present invention provides compositions comprising live replicating spumavirus vectors.

20 11. Also provided by the present invention is a method of treating a subject with a condition, wherein the condition can be a viral infection, bacterial infection, parasitic infection, proliferative disorder (eg. cancer), or a condition associated with a genetic or autoimmune disorder; comprising administering to the subject a live replicating viral vector, wherein the immunizing construct is specific for the condition.

25 12. Also provided by the present invention is a method of preventing a condition in a subject, wherein the condition can be a viral infection, bacterial infection, parasitic infection, proliferative disorder, or a condition associated with a genetic or autoimmune disorder; comprising administering to the subject a live replicating viral vector, wherein the antigen-encoding nucleic acid is specific for the condition.

30 13. Also provided are methods of using the present vector for making models and using models to study diseases and potential treatments, as well as the models themselves.

BRIEF DESCRIPTION OF THE DRAWINGS

14. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

5 15. Figure 1 shows a map of the pHSV vector detailing the location of the spumavirus *env*, *pol*, *gag*, and *bel-1 (tas)* genes. The map also indicates the presence of LTR flanking the coding region for the spumavirus genes and the presence and location of *Apal* and *NotI* restriction sites.

10 16. Figure 2 shows a detailed restriction map of the pHSV vector. The sequence of the vector is provided in SEQ ID NO: 1.

17. Figure 3 shows a linear map of pHSV with the p17/24 sequence incorporated in the vector to generate a pHSV-HIV-gag p17/24 vector. This shows the location of p17/24 within the vector.

15 18. Figure 4 shows that several TA-p17/24 clones have the desired length fragment when cut with the *Apal* and *NotI* restriction enzymes. Shown is a 1% Acrylamide gel of purified clones resulting the transfection of p17/24 into the TA cloning vector (pCR2.1). Lane 1 is the marker BSTE II. Lane 2 is an empty pHSV vector used as a negative control. Lanes 3-7 are clones 1-5.

20 19. Figure 5 shows that p24 expression can be measured by ELISA in clones 2, 3, and 4 at days 2, 4, and 6 days post infection of BHK cells. Samples were also measured for p24 expression 6 days post-infection following freeze/thaw (F/T) of the clones.

20. Figure 6 shows that p24 expression is pronounced 5 days post infection in clone 2, clone 3 and clone 4.

25 21. Figure 7 shows that expression levels of p24 are maintained at high levels even on subsequent passages. Second pass clone 2 is diluted 10-fold and dilution was used to infect BHK cells. Supernatants were removed at 3, 7, and 10 days post infection and measured via ELISA for p24 expression.

30 22. Figure 8 shows Western blot analysis of clone 2. Lane 1 is empty pHSV vector, lane 2 is mock infected BHK cells, and lane 3 is BHK cells infected with clone 2. Samples were probed with LTF001, anti-p24, or anti-HIV serum and developed.

DETAILED DESCRIPTION

22. The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following
5 description.

23. Before the present compounds, compositions, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods or specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course,
10 vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Definitions

24. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.
15 Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

25. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value.
20 Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

26. In this specification and in the claims which follow, reference will be made
25 to a number of terms which shall be defined to have the following meanings:

27. "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

Vectors

30 28. Disclosed are live replicating human spumavirus vectors suitable for human use comprising an immunizing construct, wherein the immunizing construct is inserted

in the *bet* gene. The disclosed immunizing construct can be an antigen-encoding nucleic acid.

29. Where reference is made to "antigen"-encoding nucleic acid, it is understood that in the context of the invention antigens encoded by the antigen-
5 encoding nucleic acid can include but are not limited to immunogenic or non-immunogenic peptides, polypeptides, proteins, enzymes, cytokines. These antigens can be non-human exogenous antigenic sequences from viruses, bacteria, or parasites. The antigens can also be antigenic endogenous human or human derived sequences from a condition such as a cancer. Also, peptides encoded by the antigen-encoding nucleic
10 acid can include non-antigenic sequences for the purposes of gene therapy.

30. Also disclosed are vectors of the invention, wherein the vector is pHSV or pHSV-GFP. Though at any one time reference may only be made to one of the vectors, it is an embodiment of the invention that both vectors may be used interchangeably and with equivalent results. Accordingly terms referring to either vector should be
15 understood to refer to both vectors. Similarly, reference throughout this disclosure is given to the pHSV vector containing an HIV antigen-encoding nucleic acid. It is understood that this construct may be referred to as pFOV-gag, pFOV7-gag, pFOV-7-gag, pFOV7-p17/24, pFOV-7-p17/24, pFOV-p17/24, pFOV-7GFP-gag, pFOV-7-GFP-gag, pFOV-7GFP-p17/24, pFOV-7-GFP-p17/24, pFOV-7 HIV-gag p17/24, pHSV-gag,
20 pHSV-p17/24, pHSV-GFP-gag, pHSV-GFP-p17/24 or pHSV-HIV-gag-p17/24. A vector of the invention, can have the sequence of pHSV-HIV-GAG p17/24. Thus, herein disclosed is a pHSV vector of the invention comprising SEQ ID NO: 1, wherein an antigenic construct comprising the *gag* gene of HIV-1 (SEQ ID NO: 2) has been inserted into the sequence at the unique *Apa* I site at 12816 and the unique *Not* I site at
25 13552. Said insertion resulting in the excision of nucleotides 12817-13551 of the vector and therefore partially replacing the *bet* gene of pHSV with SEQ ID NO: 2 (SEQ ID NO: 8). Therefore, also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is HIV-GAG p17/p24. Herein, p17/24 and p41 are used synonymously and are intended to refer to DNA encoding Gag or an antigenic fragment
30 of Gag. It is also herein contemplated that other genes from HIV may be used as the antigen-encoding nucleic acid in the vector of the invention. Such genes can include the *env* and *pol* genes of HIV as well as *vpu*, *vif* and *nef* genes.

31. Also disclosed are vectors of the invention, wherein the vector has the sequence of pHSV, which is defined by the restriction map shown in Figures 1, 2, and 3. It is an embodiment of the present invention that modifications can be made to the pHSV vector to incorporate additional unique restriction sites throughout the vector including but not limited to within and around the bel-2(bet), pol, gag, env, or bel-1 genes. It is also understood that such modifications may provide additional features including but not limited to reporter function, resistance to a pharmaceutical agent, or enhancers. Related, but distinct vectors are disclosed in U.S. Patent No. 5,646,032 which is incorporated in its entirety herein by reference.

32. It is well-known in the art that vaccinations can be used prophylactically for the prevention of infections as well as therapeutically for the treatment of ongoing conditions. Such infections or conditions can be but are not limited to viral infections. Thus, also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an antigen from a virus. The viral antigen can be selected from the group of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2, and Simian Immunodeficiency virus (SIV). Also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is SIV-GAG. The art is replete with examples of viral antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

33. Vaccinations are also known for the prevention of bacterial infections. Additionally, antibiotics are well-known in the art for the treatment of various bacterial infections. Herein contemplated and disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an antigen from a bacterium. The bacterial antigen
5 can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella*
10 *haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*,
15 *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species. The art is replete with
20 examples of bacterial antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

34. The vectors of the invention are not limited to bacteria and viruses. Also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an
25 antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*. The art is replete with examples of parasitic
30 antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

35. The treatment of various forms of cancer is a major concern for millions of people worldwide and the focus of much of medical research. Herein contemplated are methods of treating a cancer comprising administering to a subject the vector of the invention. Therefore, also disclosed are vectors of the invention, wherein the antigen-
 5 encoding nucleic acid is a tumor antigen. The tumor antigen can be selected from the list consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmell17, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE,
 10 GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides. Each of these antigens is known and has a coding sequence that is publically available and well-known in the art.
 15 Further cancer antigens, whether later discovered or presently know can be expressed by the present pHSV vector as described herein.

36. There are instances wherein it is advantageous to administer the vector of the invention in a pharmaceutical composition that comprises other vaccines. Pharmaceutival compositions comprising multiple vaccines can be for therapeutic or
 20 prophylactic purposes. An example Examples of such a composition is other vaccines include the mumps, measles, rubella (MMR) vaccine, and vaccines against *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species,
 25 *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*,
 30 other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*,

Neisseria gonorrhea, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species .

Specifically contemplated and disclosed are pharmaceutical compositions comprising
5 the vector of the invention and one or more additional vaccines. Also disclosed and
herein contemplated are instances in which the vector of the invention comprises more
than one antigen-encoding nucleic acid. In such a situation the vector of the invention
will produce each antigen encoded in the vector as a separate antigen.

37. There are instances in which a vector of the invention alone may not be
10 suitable for a given purpose (eg., A kit designed to screen potential drugs for the
treatment of a condition such kit, intended for use in laboratories without the
capabilities to transfect a cell-line with the vector). In such cases, cells previously
transfected with the vector of the invention are needed. Thus, also disclosed are cells
comprising the vector of the invention.

15 38. In an embodiment of the invention the antigen-encoding nucleic acid can
encode a non-antigenic sequence of DNA. This sequence can provide a functional
copy of a disrupted, mutated, disregulated or deleted gene. Examples of nucleic acids
encoding proteins that play a role in genetic disorders are known in the literature
relating to genetic disorders which is incorporated herein by reference. Methods of
20 making these cells are described and exemplified herein and in the art.

39. The ability to detect the presence of a construct can be a desirable feature
of any vector. As such, vectors are often contain a marker to show that the construct of
interest has been delivered to the subject (eg. a cell), and once delivered, is being
expressed. A marker can take the form of a gene that is detectable when expressed.
25 Thus, also disclosed are vectors of the invention further comprising a reporter gene.
One example of a reporter gene is green fluorescence protein (GFP).

Compositions

40. The invention includes a composition comprising a vector of the invention.
Disclosed are the components to be used to prepare the disclosed compositions as well
30 as the compositions themselves to be used within the methods disclosed herein. These
and other materials are disclosed herein, and it is understood that when combinations,
subsets, interactions, groups, etc. of these materials are disclosed that while specific

reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular spumavirus vector or antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) is disclosed and discussed and a number of modifications that can be made to a number of molecules including the spumavirus vector or antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) are discussed, specifically contemplated is each and every combination and permutation of spumavirus vector and antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods. Often therapeutic agents and vaccines are administered in formulations or combinations that incorporate other therapeutic modalities or necessary components for the purposes of time release, delivery, or augmentation of a response. It is understood and herein contemplated that the disclosed vectors and exogenous nucleic acid of the invention can be combined and administered with any such modality or component.

Expression systems

41. The nucleic acid vectors of the invention that are delivered to cells typically contain expression controlling systems for controlling the expression of heterologous/exogenous nucleic acid. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the

transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

Viral Promoters and Enhancers

5 42. Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus, cytomegalovirus and most preferably spumavirus, or from heterologous mammalian promoters, e.g. beta actin promoter. Each of these promoters is known and has a
10 sequence that is publicly available. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a *HindIII* E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course,
15 promoters from the host cell or related species also are useful herein. Methods of functionally linking promoters with coding sequences are well-known.

 43. Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108
20 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements
25 that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred
30 examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

44. The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or
5 alkylating chemotherapy drugs.

45. In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular
10 type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

46. It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types
15 such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

47. Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are
20 transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification
25 and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the
30 above sequences improve expression from, or stability of, the construct.

Markers

48. The viral vectors can include nucleic acid sequence encoding a marker product (reporter gene). This marker product is used to determine if the gene has been delivered to the cell and once delivered, is being expressed. Marker genes can be but
5 are not limited to the *E. Coli* lacZ gene, which encodes β -galactosidase, and green fluorescent protein.

49. In some embodiments the marker may be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin.
10 When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells
15 and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking
20 the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

50. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These
25 schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under
30 eukaryotic control to convey resistance to the appropriate drug G418 or neomycin

(geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

Treatment and Prevention Methods

51. By "treating" is meant an improvement in the disease state (i.e., viral
5 infection, bacterial infection, parasitic infection, cancer, genetic disorder, or
autoimmune disease) is observed and/or detected upon administration of a substance of
the present invention to a subject. Treatment can range from a positive change in a
symptom or symptoms of the disease to complete amelioration of the viral infection,
bacterial infection, parasitic infection, or cancer (e.g., reduction in severity or intensity
10 of disease, alteration of clinical parameters indicative of the subject's condition, relief
of discomfort or increased or enhanced function), as detected by art-known techniques.
The methods of the present invention can be utilized to treat an established viral
infection, bacterial infection, parasitic infection, or cancer. One of skill in the art would
recognize that viral infection, bacterial infection, parasitic infection, or cancer refer to
15 conditions characterized by the presence of a foreign pathogen or abnormal cell growth.
Clinical symptoms will depend on the particular condition and are easily recognizable
by those skilled in the art of treating the specific condition. Treatment methods can
include, but are not limited to therapeutic vaccinations. Thus, Disclosed are methods of
treating a subject with a condition comprising administering to the vector of the
20 invention.

52. Also disclosed are methods of the invention, wherein the condition being
treated is a viral infection. The viral infection can be selected from the list of viruses
consisting of Herpes simplex virus type-1, Herpes simplex virus type-2,
Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6,
25 Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus,
Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus,
Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus,
Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma
30 virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern
Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A,

Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

53. Also disclosed are methods of the invention, wherein the antigen-encoding
5 nucleic acid is an antigen from a virus. The viral antigen can be selected from the group of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
10 virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
15 Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

54. Also disclosed are methods of the invention, wherein the condition being
20 treated is a bacterial infection. The bacterial infection can be selected from the list of bacterium consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other
25 *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter*
30 species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other

Pseudomonas species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

55. Also disclosed are methods of the invention, wherein the antigen-encoding
5 nucleic acid is an antigen from a bacterium. The bacterial antigen can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other
10 *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus*
15 *agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*,
20 and other *Yersinia* species.

56. Also disclosed are methods of the invention, wherein the condition being treated is a parasitic infection. The parasitic infection can be selected from the list of parasites consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma*
25 *cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

57. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium*
30 *vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

58. Also disclosed are methods of the invention, wherein the condition being treated is cancer.

59. The disclosed vectors and vector containing compositions can be used to treat any disease where uncontrolled cellular proliferation occurs such as cancers. A non-limiting list of different types of cancers is as follows: lymphomas (Hodgkins and non-Hodgkins), leukemias, carcinomas, carcinomas of solid tissues, squamous cell carcinomas, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, or cancers in general.

60. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphoma, B cell lymphoma, T cell lymphoma, mycosis fungoides, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma of head and neck, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer, neuroblastoma/glioblastoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, or pancreatic cancer.

61. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is a tumor antigen. The tumor antigen can be selected from the list consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pm117, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

62. Disclosed are methods of treating a condition in a subject comprising administering to the subject the vector of the invention, wherein the condition is due to a mutated, disregulated, disrupted, or deleted gene; autoimmunity; or inflammatory diseases.

5 63. Disclosed are methods of treating a condition in a subject, wherein the condition can be selected from list consisting of cystic fibrosis, asthma, multiple sclerosis, muscular dystrophy, diabetes, tay-sachs, spinobifida, cerebral palsy, parkinson's disease, lou gehrigg disease, alzheimer's, systemic lupus erythamatosi, hemophelia, Addision's disease, Cushing's disease.

10 64. By "preventing" is meant that after administration of a substance of the present invention to a subject, the subject does not develop the symptoms of the viral, bacterial, or parasitic infection, and/or does not develop the viral, bacterial, or parasitic infection. "Preventing" or "prevention" can also refer to the ultimate reduction of an infection, condition, or symptoms of an infection, or condition relative to infections or
15 conditions in subjects that do not receive the substance. Methods of prevention can include, but are not limited to profilactic vaccination. As such, disclosed are methods of preventing an infection in a subject comprising administering to the subject the vector of the invention.

20 65. Also disclosed are methods of the invention, wherein the infection prevented is a viral infection. The viral infection can be selected from the list of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
25 virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papilomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
30 Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-

cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

66. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a virus. The viral antigen can be selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

67. Also disclosed are methods of the invention, wherein the infection prevented is a bacterial infection. The bacterial infection can be selected from the list of bacterium consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other

Hemophilus species, Clostridium tetani, other Clostridium species, Yersinia enterocolitica, and other Yersinia species.

68. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a bacterium. The bacterial antigen can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*,
5 *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus*
10 *agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

20 69. Also disclosed are methods of the invention, wherein the infection prevented is a parasitic infection. The parasitic infection can be selected from the list of parasites consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma*
25 *mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

70. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*,
30 *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

71. Also disclosed are methods of the invention, wherein the subject is a horse, cow, pig, dog, cat, mouse, monkey, human, or a cell isolated from such an animal.

Delivery of the compositions to cells

72. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, and the present viral vectors. Appropriate means for transfection, including the present viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

73. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

74. As used herein, viral vectors such as pHSV are agents that transport the disclosed antigen-encoding nucleic acids, such as p17/24 into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. Retroviral vectors especially spumavirus vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors. A preferred embodiment is a viral vector which has been engineered so as to induce the immune response of the host organism, elicited by the peptides encoded on the vector.

75. Viral vectors can have higher transfection abilities (ability to introduce genes) than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural and structural genes, a polymerase, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA.

Constructs of this type can carry large fragments of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

5 76. Spumaviruses are retroviruses. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116
10 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference. Although the present spumavirus vector is unique, the methods described for using other types of viral vectors can be useful in certain contexts. See for example U.S. Patent No. 5,646,032, which is incorporated herein for its teaching of those
15 methods.

77. A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis,
20 for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which
25 signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serves as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near
30 the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for large fragments of foreign sequence to be inserted into the viral genome, become

reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

5 78. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the
10 helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

In vivo/ex vivo

79. As described herein, the vector-containing compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the
15 subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

80. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The
20 compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known
25 for transplantation or infusion of various cells into a subject.

Nucleic Acid Delivery

81. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or RNA, or
30 the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the antibody-encoding DNA fragment is under the transcriptional regulation of a promoter, as would be well understood by one of ordinary skill in the art.

82. As one example, if the antigen-encoding nucleic acid of the invention is delivered to the cells of a subject in a spumavirus vector, the dosage for administration of spumavirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{10} to 10^{12} pfu per injection. In some cases lower dosages (eg., 10^3 , 10^4 , 10^5 , and 10^6 pfu) can be effective. A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at two, four, six month intervals (any intervening time intervals or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

83. Parenteral administration of the nucleic acid or vector of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

Delivery of pharmaceutical products

84. The vector or vector-containing compositions can be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

85. The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, subcutaneously, transdermally, extracorporeally, topically, gene gun or the like, although topical

intranasal administration or administration by inhalant is typically preferred. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. The latter may be effective when a large number of animals is to be treated simultaneously. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

86. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol., 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which

the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

10 **Pharmaceutically Acceptable Carriers**

87. The compositions, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

88. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

89. Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like.

90. The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed compositions can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, by gene gun, or transdermally.

91. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable

organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient
5 replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

92. Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional
10 pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

93. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

15 94. Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic
20 acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

Therapeutic Uses

25 95. The substances of the present invention can be delivered at effective amounts or concentrations. An effective concentration or amount of a substance is one that results in treatment or prevention of the condition (e.g. HIV or AIDS). One skilled in the art would know how to determine an effective concentration or amount according to methods known in the art, as well as provided herein. One of skill in the art can
30 utilize *in vitro* assays to optimize the *in vivo* dosage of a particular substance, including concentration and time course of administration.

96. Herein, immune modulatory substances are described. As defined immune modulation refers to any change in the immune response. This includes but is not limited to increase or decreases in the number of antigen specific plasma cells, memory B cells, memory T cells, activated CD8 T cells, cytokine production, and cytolytic killing or the maintenance of homeostatic levels of the same when the responses would otherwise be changing. The dosage ranges for the administration of the substances are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

97. For example, to evaluate the efficacy of treatment of humans with a condition, such as for example, HIV, with a substance that modulates normal immune responses to HIV, the following studies can be performed. Patients with active infection can be selected. Drug efficacy can be monitored via viral titer, antibody ELISA or ELISPOT and CD4 T cell count. Patients can be randomized to two different protocols. In one protocol, subjects can remain on initial medication and in the second protocol, subjects can have their medication tapered after receiving the substance that modulates immune responses.

98. In one embodiment, treatment can consist of either a single dosage of 0.3 mg to 0.6 mg/animal of the vector expressing a substance that treats or prevents the condition. Additionally, dosage as low as 30µg/animal to 300µg/animal can be used with intramuscular injection of infectious DNA. In one example, a DNA immunization comprising 0.3 mg of the pFOV-gag vector is administered to mouse via gene gun. After two weeks the subject is monitored using conventional assays to assess a generated immune response to p17/24 in the form of antibody production and antigen specific T cells. Having generated a response, the mouse is then challenged with an infectious dose of HIV. The mouse is then monitored for disease progression and clinical symptoms associated with HIV. Alternatively, a mouse can be infected with HIV and then given a therapeutic dose of the pFOV-gag. The animal is monitored for

changes in the viral lode, clinical progression of symptoms, as well as immune responses in comparison to non treated control animals. In a further example, a DNA immunization comprising 0.05mg (50µg) of the pFOV-gag vector is administered to mouse via intrauscular injection of infectious DNA. After two weeks the subject is
5 monitored using conventional assays to assess a generated immune response to p17/24 in the form of antibody production and antigen specific T cells. Having generated a response, the mouse is then challenged with an infectious dose of HIV. The mouse is then monitored for disease progression and clinical symptoms associated with HIV. Alternatively, a mouse can be infected with HIV and then given a therapeutic dose of
10 the pFOV-gag. The animal is monitored for changes in the viral lode, clinical progression of symptoms, as well as immune responses in comparison to non treated control animals. The art of determining dosage for an animal based on size is well known. It is understood that a skilled artisan would be able to determine the proper dosage of a substance for an animal based on the dosage of the same substance
15 administered to another animal of similar or different size. For example, whereas a 200g mouse would receive a 50µg dose of the immunization, a 150lb (68kg) human would be administered an immunization comprising about 17mg of the vector.

99. Disclosed are methods of detecting the expression of the vector of the invention comprising using a first antibody to the antigen to measure protein expression
20 in a quantitative or qualitative way, further comprising detecting the first antibody directly via a colorimetric measurement produced through the use of a substrate and a conjugated antibody or indirectly via a first antibody to the antigen which in turn is bound by a second antibody which is conjugated and will result in a colorimetric measurement when combined with a substrate.

25 100. Also disclosed are methods of the invention, wherein the antigen is detected by placing an aliquot of the vector of the invention in a lane on a gel and probing the gel for the antigen.

101. Disclosed are methods of detecting the expression of the vector of the invention comprising using a fluorescently labeled first antibody specific for the
30 antigen and visualizing the antigen using a flow cytometer or fluorescence microscope.

102. Also disclosed are methods of the invention, wherein the first antibody is not fluorescently labeled, but a target for a second antibody with a fluorescent label.

103. Disclosed are methods of detecting the expression of the vector of claim the invention comprising using cytolitic killing assay to assess activity.

104. Disclosed are methods of detecting the vector of the invention further comprising obtaining a sample from a subject comprising a tissue biopsy or removal of
5 blood or bone marrow.

Screening Methods

105. Also provided by the present invention is a method of screening a substance for effectiveness in treating or reducing the severity of the condition (e.g., HIV infection) comprising: a) obtaining an animal having the condition or
10 characteristic (e.g., symptom) of the condition; b) administering the substance to an animal having one or more characteristics of the condition; and assaying the animal for an effect on the condition, thereby identifying a substance effective in reducing the condition.

106. The ability of a substance to reduce the severity of a condition can be
15 determined by evaluating the histological and clinical manifestations, of the animal with condition before and after administration of the substance of interest and quantitating the amount of reduction of the condition.

107. The animal in which the condition or characteristic (e.g., symptom) of the condition is produced can be any mammal and can include but is not limited to
20 mouse, rat, guinea pig, hamster, rabbit, cat, dog, goat, monkey, and chimpanzee. The condition or characteristic (e.g., symptom) of the condition can be produced in the animal by any method known in the art. For example, HIV can be produced by introducing into the animal (eg., chimpanzee infected with HIV or rhesus macaques or nemestrina macaques infected with an HIV-1 env on an SIV backbone. Pullium, JK, et.
25 al., J. Infectious Dis. 183:1023, 2001) an infectious amount of HIV.

108. The present invention also provides a method of screening for a substance effective in preventing the condition (e.g., HIV infection) comprising: a) administering the substance to an animal susceptible to the condition; b) subjecting the animal to treatment that will induce the condition or characteristic (e.g., symptom) of
30 the condition; and c) assaying cells from the animal for an change in immune responses as compared to an the immune responses in a control animal having the condition in the

absence of the substance identifies a substance that is effective in preventing the condition.

109. For example, the methods of measuring the amount of p17/24 or other HIV antigen in an animal include, but are not limited to, ELISA, PCR, FACS analysis, reverse-transcriptase-polymerase chain reaction and ELISPOT, Northern blots, Southern blots, and Western blots.

110. A model for use in screening for substances effective in treating or preventing a disease comprising an animal capable of manifesting a characteristic of the disease is provided, wherein the animal has been administered the vector of the invention.

111. A method of making the model of the invention comprising obtaining administering to an animal capable of manifesting a characteristic of the disease and administering to said animal the vector of claim 1a vector of the invention which encodes an antigen associate with the disease.

112. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) administering the substance to the model of the invention; and b) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

113. A method of screening for a substance effective in preventing a disease associated with an immunizing construct comprising: a) administering a the vector of the invention to a subject; b) subjecting the subject to treatment that will induce the disease or characteristic (e.g., symptom) of the disease; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in preventing the disease.

114. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) subjecting the subject to treatment that will induce the disease or characteristic (e.g., symptom) of the disease; b) administering a the vector of the invention to a subject; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control

subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

Sequence similarities

115. It is understood that as discussed herein the use of the terms homology
5 and identity mean the same thing as similarity. Thus, for example, if the use of the
word homology is used between two non-natural sequences it is understood that this is
not necessarily indicating an evolutionary relationship between these two sequences,
but rather is looking at the similarity or relatedness between their nucleic acid
sequences. Many of the methods for determining homology between two
10 evolutionarily related molecules are routinely applied to any two or more nucleic acids
or proteins for the purpose of measuring sequence similarity regardless of whether they
are evolutionarily related or not.

116. In general, it is understood that one way to define any known variants
and derivatives or those that might arise, of the disclosed genes and proteins herein, is
15 through defining the variants and derivatives in terms of homology to specific known
sequences. This identity of particular sequences disclosed herein is also discussed
elsewhere herein. In general, variants of genes and proteins herein disclosed typically
have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87,
88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence
20 or the native sequence. Those of skill in the art readily understand how to determine
the homology of two proteins or nucleic acids, such as genes. For example, the
homology can be calculated after aligning the two sequences so that the homology is at
its highest level.

117. Another way of calculating homology can be performed by published
25 algorithms. Optimal alignment of sequences for comparison may be conducted by the
local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by
the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443
(1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad.
Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms
30 (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package,
Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

118. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material
5 related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

119. For example, as used herein, a sequence recited as having a particular
10 percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not
15 have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80
20 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using
25 each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

Nucleic acids

120. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example HIV-1 *gag*, as
30 well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for

example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

Sequences

121. There are a variety of sequences related to the *gag* gene that are publicly available (eg., Genbank Accession Number: L03707), these sequences and others are herein incorporated by reference in their entirety as well as for individual subsequences contained therein.

122. One particular sequence set forth in *gag* that is publicly available and having Genbank accession number L03707 is used herein, as an example, to exemplify the disclosed compositions and methods. It is understood that the description related to this sequence is applicable to any sequence related to *gag* unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of *gag*). Primers and/or probes can be designed for any *gag* sequence given the information disclosed herein and known in the art.

Peptides/ Protein variants

123. There are numerous variants of protein antigens that are antigenic. For example, there are variants of the *gag* protein and protein that are known and herein contemplated. In addition to the known functional HIV-1 *gag* strain variants there are derivatives of the *gag* proteins which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein

derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1: Amino Acid Abbreviations

Amino Acid	Abbreviations
Alanine	Ala, A
Allosoleucine	AlIle
Arginine	Arg, R
Asparagine	Asn, N
aspartic acid	Asp, D
Cysteine	Cys, C
glutamic acid	Glu, E
Glutamine	Gln, Q
Glycine	Gly, G
Histidine	His, H
Isoleucine	Ile, I
Leucine	Leu, L

Amino Acid	Abbreviations
Lysine	Lys, K
Phenylalanine	Phe, F
Proline	Pro, P
pyroglutamic acidp	Glu
Serine	Ser, S
Threonine	Thr, T
Tyrosine	Tyr, Y
Tryptophan	Trp, W
Valine	Val, V

TABLE 2: Amino Acid Substitutions	
Original Residue	Exemplary Conservative Substitutions, others are known in the art.
	Ala; Ser
	Arg; Lys, gln
	Asn; Gln; his
	Asp; Glu
	Cys; Ser
	Gln; Asn, lys
	Glu; Asp
	Gly; Pro
	His; Asn; gln
	Ile; Leu; val
	Leu; Ile; val
	Lys; Arg; gln;
	Met; Leu; ile
	Phe; Met; leu; tyr
	Ser; Thr
	Thr; Ser
	Trp; Tyr
	Tyr; Trp; phe
	Val; Ile; leu

124. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic

residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

125. The replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the antigenic polypeptides encoded and expressed by the vectors provided herein.

126. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

127. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

128. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:3 sets forth

a particular sequence of a *gag* protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. It is understood that those of skill in the art will recognize variants of a disclosed protein as being a variant. Particularly, there are numerous naturally occurring variants of viral (e.g., HIV) antigens. For example, it is understood that those of skill in the art would recognize that the particular *gag* protein disclosed in SEQ ID NO: 4 is a *gag* protein from a different HIV-1 isolate from the HIV-1 *gag* protein in SEQ ID NO: 3 and therefore a variant that can be used with equivalent results. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

129. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

130. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

131. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

132. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein

sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO: 3 is set forth in SEQ ID NO: 2. In addition, for example, a disclosed conservative derivative of SEQ ID NO: 4 is shown in SEQ ID NO: 5, where the isoleucine (I) at position 19 is changed to a valine (V). It is understood that for this mutation all of the nucleic acid sequences that encode this particular derivative of the *gag* gene of the LAI strain of HIV-1 are also disclosed. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular *gag* from which that protein arises is also known and herein disclosed and described.

Computer readable mediums

133. It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

Methods of making the compositions

134. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

5 Nucleic acid synthesis

135. For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for
10 example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Bioscience, Burlington, MA or ABI Model
15 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

20 136. Disclosed are cells produced by the process of transforming the cell with any of the disclosed nucleic acids. Disclosed are cells produced by the process of transforming the cell with any of the non-naturally occurring disclosed nucleic acids.

137. Disclosed are any of the disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the non-naturally
25 occurring disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the disclosed peptides produced by the process of expressing any of the disclosed non-natural nucleic acids.

138. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed
30 are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal

with any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

139. Also disclose are animals produced by the process of adding to the animal any of the cells disclosed herein.

5 **Methods of using the compositions as research tools**

140. The disclosed vectors, compositions and methods can be used for targeted gene disruption and modification in any animal that can undergo these events. Gene modification and gene disruption refer to the methods, techniques, and compositions involved in the selective removal or alteration of a gene or stretch of
10 chromosome in an animal, such as a mammal, in a way that propagates the modification through the germ line of the mammal. In general, a cell is transformed with a vector which is designed to homologously recombine with a region of a particular chromosome contained within the cell, for example, as described herein. This homologous recombination event can produce a chromosome which has
15 exogenous DNA introduced, for example in frame, with the surrounding DNA. This type of protocol allows for very specific mutations, such as point mutations, to be introduced into the genome contained within the cell. Methods for performing this type of homologous recombination are disclosed herein.

141. One of the preferred characteristics of performing homologous
20 recombination in mammalian cells is that the cells should be able to be cultured, because the desired recombination events occur at a low frequency.

142. Once the cell is produced through the methods described herein, an animal can be produced from this cell through either stem cell technology or cloning technology. For example, if the cell into which the nucleic acid was transfected was a
25 stem cell for the organism, then this cell, after transfection and culturing, can be used to produce an organism which will contain the gene modification or disruption in germ line cells, which can in turn be used to produce another animal that possesses the gene modification or disruption in all of its cells. In other methods for production of an animal containing the gene modification or disruption in all of its cells, cloning
30 technologies can be used. These technologies generally take the nucleus of the transfected cell and either through fusion or replacement fuse the transfected nucleus with an oocyte which can then be manipulated to produce an animal. The advantage of

procedures that use cloning instead of ES technology is that cells other than ES cells can be transfected. For example, a fibroblast cell, which is very easy to culture can be used as the cell which is transfected and has a gene modification or disruption event take place, and then cells derived from this cell can be used to clone a whole animal.

5 143. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in
10 the sentence or section in which the reference is cited.

 144. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention
15 disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

Examples

 145. The following examples are put forth so as to provide those of ordinary
20 skill in the art with a complete disclosure and description of how the vectors, compounds, compositions, and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors
25 and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Example 1

 146. In order to obtain the p17/p24 fragment of the HIV-1 gag gene, pSX
30 plasmid containing the gag gene was subjected to 30 cycles of PCR using forward Apa1-p17 5' primer and reverse Not1-p24 3' primer. PCR product was cloned into a TA cloning vector (pCR2.1 Invitrogen; Carlsbad, CA) and expanded. Fig. 1 shows a

map of the empty pHSV vector. The vector contains the viral envelope gene *env* as well as its structural gene *gag* and viral polymerase gene *pol*. The vector also possesses a transactivator (TAS) gene *bel-1* and 2 LTR that flank the coding region of the vector. An inspection of the vector SEQ (SEQ ID NO: 1) shows where various restriction sites are located on the vector. A complete listing of the sites is found in Fig. 2 and these sites are shown in SEQ ID NO: 1.

147. Inspection of the restriction map revealed *Apal* and *NotI* unique restriction sites located around the BET (Bel-2) gene at 13522 and 12816 respectively. As such, the BET gene was chosen as the site for integration of the exogenous nucleic acid (Figure 3). The p17/p24 were excised from the TA cloning vector using *Apal* and *NotI* restriction enzymes. Similarly, the empty pHSV vector was cut with *Apal* and *NotI*.

148. The p17/p24 segment of *gag* (789 (start of p17) – 1876 (end of p24)) was ligated to pHSV and the pHSV-GAG was transformed into *E. coli* via electroporation. The bacteria was plated onto high Amp plates with the pHSV conferring *ampR* between the LTRs on the approximate 3kb plamid. Seven colonies were chosen and checked for insertion and proper orientation. Figure 4 shows that clone 2, 3, 4 when cut with *Apal* and *NotI* possessed a 1 kb fragment. This indicates that the p17/p24 fragment is intact and properly oriented.

149. Knowing that the construct was integrated into the vector, the next objective was to examine expression levels of the antigen. Integration is meaningless if the desired protein is not expressed. To rest expression of p17/p24, the loaded pHSV vector was transfected using lipofectamine into BHK cells and supernatants were collected at various time points post transfection. ELISA testing for p24 expression was conducted on the collected supernatants (Fig. 5).

150. Following transfection, clone 2 had clearly measurable p24 expression. The p24 expression level for clone 2 was increased at each time point after day 2, clones 3 and 4 showed no measurable expression of p24 until day 5 post transfection. Subsequent Elisa data revealed that 5 days post transfection clones 2, 3, and 4 could produce as much as 500 ng/ml, 1000ng/ml, and 800ng/ml of p24 respectively (Fig. 6). At the same time *in vitro* cytopathology was assessed. Cells from the day 2 cultures

had very little CPE. However, by day 5 CPE was readily observed. The presence of CPE shows that the p24 activity was associated with the replicating viral vector.

151. Fig. 7 shows p24 expression in clone 2 after 2nd pass. Supernatants were collected at 3, 7 and 10 days after infection with diluted virus. At day 3 no p24 is
5 observed. However, by day 7 1000ng/ml of p24 is observed at the 10⁻² dilution, which is confirmed at the 10⁻³ dilution with a measurement of 100ng/ml. By day 10, the 10⁻² to 10⁻⁴ dilutions all had approximately 200ng/ml of p24. As such, day 7 undiluted virus would yield 10⁵ ng/ml p24 and at day 10 the secretion level ranges from 2x10⁴ to 2x10⁶ ng/ml of p24. This is an extremely high expression level and indicates likely success *in*
10 *vivo* as this vector is resulting in expression comparable to the highest 25% of vectors.

152. As a verification that p24 Elisa data was actually measuring p17/p24 (p41) and not just p24, western blotting was performed (Fig. 8). Supernatants from BHK cells infected with clone 2 pHSV-gag, empty pHSV, or mock infected (infected with PBS) were run on a polyacrylamide gel and probed for p24, HIV, or LTF001 as a
15 positive control. LTF001 antibody bound to all 3 lanes. However, bands were apparent only in the lane for clone 2 when either anti-p24 or anti-HIV was used as a probe. The resulting bands show the smallest band to be 41kd, which corresponds to the size of p17/p24.

153. Histological observations were made from *in vitro* culture of pHSV in
20 BHK cells. Cells infected with pHSV-gag showed CPE; however, this CPE is not usually observed *in vivo*.

Methods

Cloning and PCR

154. The p17 and p24 segments of the LAI strain of HIV-1 gag were cloned
25 into a pHSV spumavirus vector. Briefly, pSX plasmid containing the LAI strain HIC-1 gag gene was obtained from the HIV repository. The pSX plasmid was used as a template for PCR amplification and cloning of p17 and p24. A forward Apa1-p17 5' primer TCC GGG CCC GGA ATG CCT ATA GTC CAG AAC ATC C (SEQ ID NO: 6) and a reverse Not1-p24 3' primer GCG GCC GCG TTT TGA GAA CGA AAT
30 ACC GG (SEQ ID NO: 7) were used to amplify p41. The amplified segment was cloned into a TA cloning vector (pCR2.1; Invitrogen Carlsbad, CA) and expanded.

Restriction digest of the TA-p41 vector with Apa1 and Not1 removed p17/24 from the vector. The p41 segment was then inserted into pHSV within the *bet* (*bel-2*) gene.

Transformation

155. The pHSV vector and p17/24 antigen were ligated together with ligase
5 for 16hrs at 4C. Following ligation, pHSV-gag was electroporated into E. coli and incubated for 1hr at 37C in 2xyT broth media. The samples are then plated on high Ampicillin plates and incubated overnight at 37C. Colonies growing on the high Amp plates were selected and transferred to 2xyT broth media overnight at 37C. Minipreps were performed on the overnight cultures. Plasmids were cut with Apa1 and Not1 and
10 aliquots were run on 1% agarose gel.

ELISA and Western Blot

156. ELISA and Western blot were performed using techniques commonly used in the art.

Example 2

157. Animal subjects can be used to screen the effectiveness of a pHSV
15 vector and an antigen-encoding nucleic acid. Additionally, animal subjects may be used to study the vector-antigen combination's ability to prevent or treat a condition. The vector can also be used to induce a condition in an animal that is associate with a disease, and that animal can then be used to study the disease/condition or to study
20 potential treatments for the disease/condition. Such conditions can be infections resulting from viruses, bacteria, or parasites; autoimmune reactions including inflammatory diseases, asthma, systemic lupus erythamatosi, muscular dystrophy, or multiple sclerosis, diabetes, tay-sachs, spinobifida, cerebral palsy, parkinson's disease, lou gehrigg disease, alzheimer's, hemophelia, Addision's disease, Cushing's disease; or
25 cancer. Animals can include but are not limited to mice, rats, pig, dog, monkey, chimpanzee, and human.

158. Mice are injected with a sub-immunizing dose of pHSV-gag. 0.3mg/
animal of pHSV-gag are administered via gene gun. This same immunizing dose may be administered by an alternative route such as intramuscular, intravenous,
30 intraperitoneal, intracranial, or subcutaneous. Mice are then monitored and starting at 15 days post immunization mice are bled every three days to day 30 post immunization. Blood samples are collected for serum extraction and for the isolation of peripheral

blood lymphocytes (PBL). Serum samples can be used immediately for ELISA assays to look for antibody to the antigen encoded in the vector or for cytokine increases following immunization. Samples can also be frozen and stored at -80°C for testing at a later time.

5 159. Lymphocytes are removed from whole blood and diluted in media (e.g. RPMI 1640, DMEM, MEM, or EMEM) with 10% serum. PBL are then stained for antigen specific T cells using antigen specific MHC class I and MHC class II tetramers to be visualized via flow cytometry. Simultaneously or separately PBL are stained with surface markers to look at activation characteristics of the cells and other surface
10 phenotypes. Some examples of the antibodies used are CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45RA, CD45RO, CD62L (L-selectin), CD69 and Bcl-2. Additionally, PBL can be stained for the production of intracellular cytokines such as IFN- γ , IL-4, TNF- α , IL-2, and IL-10 following stimulation with antigen. Lymphocytes can also be used in an ELISPOT assay for cytokine production following stimulation
15 with specific antigen or CTL assay to assess killing activity.

 160. All of the stains and assays allow for the assessment of cell-mediated responses to the priming antigen. Tetramer staining allows for the enumeration of antigen specific T cells of a known specificity. This coupled with surface markers details the activation state of the antigen specific cells. Tetramer positive T cells
20 (CD8+/CD3+, CD3+, or CD3+/CD4+) possess various surface markers that reveal the activation state of the cells. By multiplying percentages obtained from the analysis of the staining by the number of cells, the exact number of cells exhibiting any characteristic can be obtained. Exposure to an antigen creates cells that express high levels of CD11a, and CD44. Once these levels are increased on a cell, they will not
25 decrease, and in conjunction with tetramer stains and other surface markers can lead to the identification of memory T cells. CD43 and CD69 both increase during activation, but decrease following clearance of the priming antigen. L-selectin will decrease on the surface of activated cells and increase over time to naïve levels. Bcl-2 also provides a measure of the activation level of cells as activated cells will have decreased Bcl-2
30 levels relative to naïve T cells indicating a susceptibility to apoptosis whereas memory cells will have increased levels of Bcl-2.

161. ELISPOT and intracellular cytokine stains allow the enumeration of cytokine secreting antigen-specific cells following stimulation. This can be paired with the number of tetramer positive cells to determine what portion, if not all, of the T cells are functional.

5 162. CTL assays determine the ability for antigen specific T cells to kill cells expressing the antigen. The assay reads on the cytolytic activity of T cells as determined by release of a chemiluminescent marker or radioactive material in labeled target cells.

10 163. Like the cell-mediated responses, humoral responses may be measured in harvested PBL. PBL maybe stained for anti-mouse IgG, CD45R (B220), PNA, and CD138 (syndecan-1) to look at the activation of B cells or the number of plasma cells. Plasma cells, the effector arm of the humoral immune system are syndecan-1+, and B220-. Memory B cells will be IgG+, B220+, and PNA+, the presence of PNA positive cells indicates transition through a germinal center reaction. Additionally,
15 PBL may be used to enumerate the number of antigen specific plasma cells on a plasma cell elispot or the number of antigen specific B cells on a memory B cell assay.

164. ELISA data can be combined with the humoral immunity data from the PBL to assess the effect of the immunization on the mouse.

20 165. Mice that have measurable responses can be boosted and responses monitored. However, direct challenge is necessary as it indicates the ability to mount a protective immune response. Mice that do not have an immune response or mice that have an immune response that but need to be boosted can receive second and third immunizations by the same or different route than the priming immunization over the next few months.

25 166. As mice cannot be infected with a lentivirus, monkeys (e.g., rhesus macaques) are used for challenge experiments. Monkeys can receive a similar construct (pHSV-SIV-gag) which produces similar levels of gag in vitro. Monkeys are inoculated with the pHSV-SIV-gag DNA, and after several weeks, they can be challenged with an infectious SIV. The animals can then be observed for protection
30 from infection or disease.

167. For a challenge, monkeys are given an infectious dose of an antigen (e.g. SIV) or stimulated in such a way as to induce a condition to be prevented. Challenged

monkeys can be assessed in the same way as immunized mice with additional assays used to look at antigen load following challenge and gross pathological and histological assessments made from tissue biopsy or necropsy.

168. Monkeys can also be given an infectious dose of an antigen (e.g. SIV) or stimulated in such a way as to create a condition to be treated. Once the condition is established, monkeys are given a therapeutic dose of the immunizing antigen and monitored as to the effects on the condition. A successful therapy will not necessarily clear the condition or infection, but may slow or stop progression. The same assays to those used for prophylactic immunization are used to characterize the responses

10 **Methods**

Flow cytometry

169. Cells are suspended at a concentration that is appropriate according to the antibody manufacturers instructions in FACS buffer (2% FCS (or BSA) in PBS (0.2% NaN₃ can also be added)). Antibodies (CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45R (B220), CD45RA, CD45RO, CD62L (L-selectin), CD69, CD138, PNA, anti-mouse IgG) are added to the cells according to the manufacturers instructions and incubated for 30min at 4C in the dark. Cells are washed 3X in FACS Buffer. 1 wash comprises centrifuging the cells at 800rpm for 3min to pellet the cells, removing the media, and resuspending the cells. After the third wash, cells are resuspended in 2% PFA in PBS. Staining is analyzed on a FACSCalibur flow cytometry instrument (Beckton-Dickenson) or other suitable cytometer.

Intracellular cytokine staining.

170. Cells are stimulated for 5hrs in the presence of antigen and BFA (meninsin may be substituted for BFA) at 37C (this stimulation is not needed for Bcl-2 staining). After the 5hr incubation, cells are centrifuged and the media removed and resuspended in FACS buffer (2% FCS (or BSA) in PBS (0.2% NaN₃ can also be added)). Antibodies (CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45R (B220), CD45RA, CD45RO, CD62L (L-selectin), CD69, CD138, PNA, anti-mouse IgG) are added to the cells according to the manufacturers instructions and incubated for 30min at 4C in the dark. Cells are washed 3X in FACS Buffer. 1 wash comprises centrifuging the cells at 800rpm for 3min to pellet the cells, removing the media, and resuspending the cells. After the third wash, cells are resuspended in cytofix/cytoperm

solution for 20min at 4C. Cells are then washed 3X in a permwash solution and resuspended at a concentration that is appropriate according to the antibody manufacturers instructions in permwash. Antibodies (IFN- γ , TNF- α , IL-2, IL-4, IL-10, and Bcl-2) are added to the cells according to the manufacturers instructions and

5 incubated for 30min at 4C in the dark. Cells are washed 3X in PermWash and 2X in FACS Buffer. After the second FACS Buffer wash, cells are resuspended in 2% PFA in PBS. Staining is analyzed on a FACSCalibur flow cytometry instrument (Beckton-Dickenson) or other suitable cytometer. Intracellular kits are commercial available through (Beckton-Dickenson).

10 ELISA

171. 96-well ELISA plates are coated with antigen overnight at 4C. Plates are blocked with a suitable blocking media containing 10% FCS for 1-2hrs at RT. Plates are resuspended in a known volume of an ELISA diluent (e.g., PBS+10% FCS) and serum is added to the plate and incubated at RT for 1.5hrs. Plates are washed with

15 PBS+0.1%Tween 3X and 100 μ l of antibodies (e.g., HRPO conjugated anti-mouse IgG) diluted 1:1000 in ELISA diluent is added to each well. Plates are incubated for 1.5hrs at RT and then washed 3x with PBS+0.1%Tween. Plates are then coated with 100 μ l of a chromagen substrate (e.g., o-phenyldiamine + 3% H₂O₂ in citrate buffer (Sodium citrate in H₂O pH=5) and incubated for 1hr in the dark. The reaction is stopped by

20 adding 100 μ l of 1N HCl. Plates are read in a ELISA plate reader.

Plasma cell ELISPOT

172. 96-well filter (ELISPOT) plates are coated with antigen overnight. Plates are washed 1X in PBS-0.1%Tween and 3X in PBS. Plate are blocked 1-2hrs with media + 10% FCS. After blocking, media is removed and 100 μ l of media are

25 added to each well. Effector cells (cells containing lymphocytes from an immunized animal (e.g., PBL, splenocytes, hepatocytes, and bone marrow) are added to the plate and serial dilutions are made. Plates are incubated for 5hrs at 37C. After the 5hr incubation, plates are washed 3X in PBS and 3X in PBS+0.1% Tween. Plates are then coated with 100 μ l PBS+0.1% Tween+1% FCS and biotinylated Ab at 1/100

30 concentration overnight at 4C. After incubation plates are washed 4X in PBS+0.1%Tween and incubated for 1hr at RT in PBS+0.1%Tween+1%FCS containing HRP-avidinD at 1/1000. Plates are washed 3X with PBS and a suitable chromagen

substrate is added (e.g., AEC). Plates are allowed to develop for 8-15min and the reaction is stopped with washing the plates with water. Spots are visualized and counted using a dissection microscope or ELISPOT plate reader.

Cytokine ELISPOT

- 5 173. The day prior to the assay coat each well with 50 μ l 10 μ g/ml purified anti-cytokine antibody (e.g., IFN- γ , TNF- α , IL-2, IL-4, IL-10). Incubate overnight at 4 °C. Wash plates 4 times with 200 μ l PBS. Block wells with 200 μ l 10%FCS complete medium, incubate at room temperature for at least 1 hour. Add effector cells to the top wells and make serial dilutions. Discard 100 μ l of cells from the last dilution. Add the
- 10 stimulating antigen and IL-2 to the wells. Incubate plates at 37 °C, 5% CO₂ for 16-40 hours (use level to ensure that plates stay even horizontally). Discard cells and wash 5 times with PBS-Tween). Add 100 μ l 2 μ g/ml biotinylated-anti-cytokine antibody (e.g., IFN- γ , TNF- α , IL-2, IL-4, IL-10) diluted in PBS-Tween. Incubate at room temperature for 2 hours or overnight at 4C. Wash plate 5 times with PBS-Tween, 200 μ l each wash.
- 15 Add 100 μ l 1:1000 Streptavidin –HRP, incubate 1 hour at room temperature. (avoid high background by not exceeding 1 hour incubation). Wash wells 5 times with PBS-Tween. Add 100 μ l chromagen substrate per well, incubate at room temperature for 10-20 minutes in dark (at 10 minutes, observe the color developed, if the color is not fully intense, incubate few more minutes and double check). Wash plates under
- 20 running tap water and air-dry plates (in hood with light turned-off). Count and record numbers of spots/well by ELISPOT plate reader or stereomicroscope (20x).

CTL

174. Direct ex-vivo cytolytic killing is measured in a 5hr ⁵¹Cr release assay. Briefly, target cells are labeled with ⁵¹Cr for 1hr at 37C. Following the 1hr incubation,
- 25 cells are centrifuged for 8min at 1200rpm to pellet the cells. The media is poured off and the pellet resuspended in fresh media. Cells are centrifuged and resuspended two additional times. After the third spin, cells are resuspended in an appropriate amount of media to attain the desired effector to target ration (e.g., a 50:1 effector: target ratio with effector cells at a concentration of 1×10^7 / ml would require target cells to be at a
- 30 concentration of 2×10^5 /ml), and contacted with antigen. Effector cells (cells containing lymphocytes from an immunized animal (e.g., PBL, splenocytes, hepatocytes, and bone marrow) are contacted with the target cells for 5-6hrs at 37C. Cells are pelleted by

centrifugation and supernatants harvested. ^{51}Cr release is measured on an appropriate instrument. Percent killing is (experimental release-spontaneous release / maximum release – spontaneous release) x 100.

- 5 175. Secondary killing to measure memory cell responses to antigen can also be measured. In this method, effector cells are incubated for 6 days in the presence of antigen stimulation and then used in a cytolytic assay as described above.

Example 3

- 10 176. In accordance with the methods described above the pHSV vector comprising an SIV-Gag-p17/p27 construct was made. This vector is useful to establish a model for the study of the effectiveness of a particular treatment or prophylactic vaccine. For example, such a model can comprise a system to establish a foamy live viral vector for vaccine use. The pHSV with an SIV gag (p17,p27) insert has been tested in vitro and shown to express similar levels of SIV gag as the HIV gag engineered vector. This SIVgag live viral vector can be used to inoculate rhesus macaques in order to determine protective immune responses that can develop following in vivo expression of the vector gene products including the SIV gag protein. Inoculated animals can then be challenged with wild type SIV to further determine any potential vaccine induced efficacy by studying primary (sterilizing immunity) and secondary (time to morbidity) end points.
- 15

CLAIMS

What is claimed:

1. A live replicating human spumavirus vector (pHSV) suitable for human use comprising an immunizing construct, wherein the immunizing construct partially replaces the *bet* gene.
2. The vector of claim 1, wherein the vector has the sequence of SEQ ID NO:1.
3. The vector of claim 1, further comprising a reporter gene.
4. The vector of claim 3, wherein the reporting gene is GFP.
5. The vector of claim 1, wherein the immunizing construct is an antigen encoding nucleic acid.
6. The vector of claim 5, wherein the antigen-encoding nucleic acid encodes a viral antigen.
7. The vector of claim 6, wherein the viral antigen is an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.
8. The vector of claim 7, wherein the viral antigen is HIV-GAG p17/p24.
9. The vector of claim 7, wherein the immunizing construct is SIV-GAG.
10. The vector of claim 5, wherein the antigen-encoding nucleic acid encodes a bacterial antigen.
11. The vector of claim 10, wherein the bacterial antigen is an antigen from a bacterium selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis*

strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

12. The vector of claim 5, wherein the peptide encoding nucleic acid encodes a parasitic antigen.

13. The vector of claim 12, wherein the parasitic antigen is an antigen from a parasite selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

14. The vector of claim 5, wherein the peptide encoding nucleic acid encodes a cancer antigen.

15. The vector of claim 14, wherein the cancer antigen is an antigen from a cancer selected from the group consisting of lymphomas (Hodgkins and non-Hodgkins), B cell lymphoma, T cell lymphoma, leukemias, myeloid leukemia, carcinomas, carcinomas of solid tissues, squamous cell carcinomas of mouth, throat, larynx, lung, head and neck, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, mycosis fungoides, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer,

neuroblastoma/glioblastoma, ovarian cancer, liver cancer, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, or pancreatic cancer.

16. The cancer antigen of claim 15, wherein the antigen is selected from the list of cancer antigen consisting of mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmell7, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

17. A cell comprising the vector of claim 1.

18. A method of detecting the expression of the vector of claim 1, comprising obtaining a sample containing the vector, contacting the sample with an antibody directed to antigen encoded by the immunizing construct, and detecting antigen bound to the antibody, the presence of bound antigen indicating expression of the vector.

19. The method of claim 18, wherein the sample is blood from a subject.

20. The method of claim 18, wherein the sample is tissue biopsy from a subject.

21. A method of treating a subject with a condition comprising administering to the subject the vector of claim 1.

22. The method of claim 21, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma

virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

23. The method of claim 21, wherein the condition is associated with bacterial infection, and the immunizing construct encodes an antigen from a bacterium selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

24. The method of claim 21, wherein the condition is associated with parasitic infection, and the immunizing construct encodes an antigen from a parasite selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

25. The method of claim 21, wherein the condition is cancer, and the immunizing construct encodes a tumor antigen from the list consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast

cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmel17, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

26. A method of preventing a condition in a subject comprising administering to the subject the vector of claim 1.

27. The method of claim 26, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

28. The method of claim 26, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria*

monocytogenes, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

29. The method of claim 28, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

30. A method of making a model for use in screening for substances effective in treating or preventing a disease associated with an immunizing construct, comprising administering the vector of claim 1 to an animal capable of manifesting a characteristic of the immunizing construct.

31. A model made in accordance with the method of claim 30.

32. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) administering the substance to the model of claim 31; and b) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

33. A method of screening for an immunizing construct effective in preventing a disease associated with an immunizing construct comprising: a) administering the vector of claim 1 to a subject; b) subjecting the subject to treatment that will induce the disease or characteristic of the disease; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an

improvement in the course of the disease in the presence of the substance identifies a substance that is effective in preventing the disease.

34. A method of screening for an immunizing construct effective in treating a disease effective in treating a disease associated with an immunizing construct comprising: a) subjecting the subject to treatment that will induce the disease or characteristic of the disease; b) administering the vector of claim 1 to a subject; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

35. The method of claims 21-34, wherein the subject is a cell.

36. The method of claims 21-34, wherein the subject is a mouse.

37. The method of claims 21-34, wherein the subject is a monkey.

38. The method of claims 21-34, wherein the subject is a human

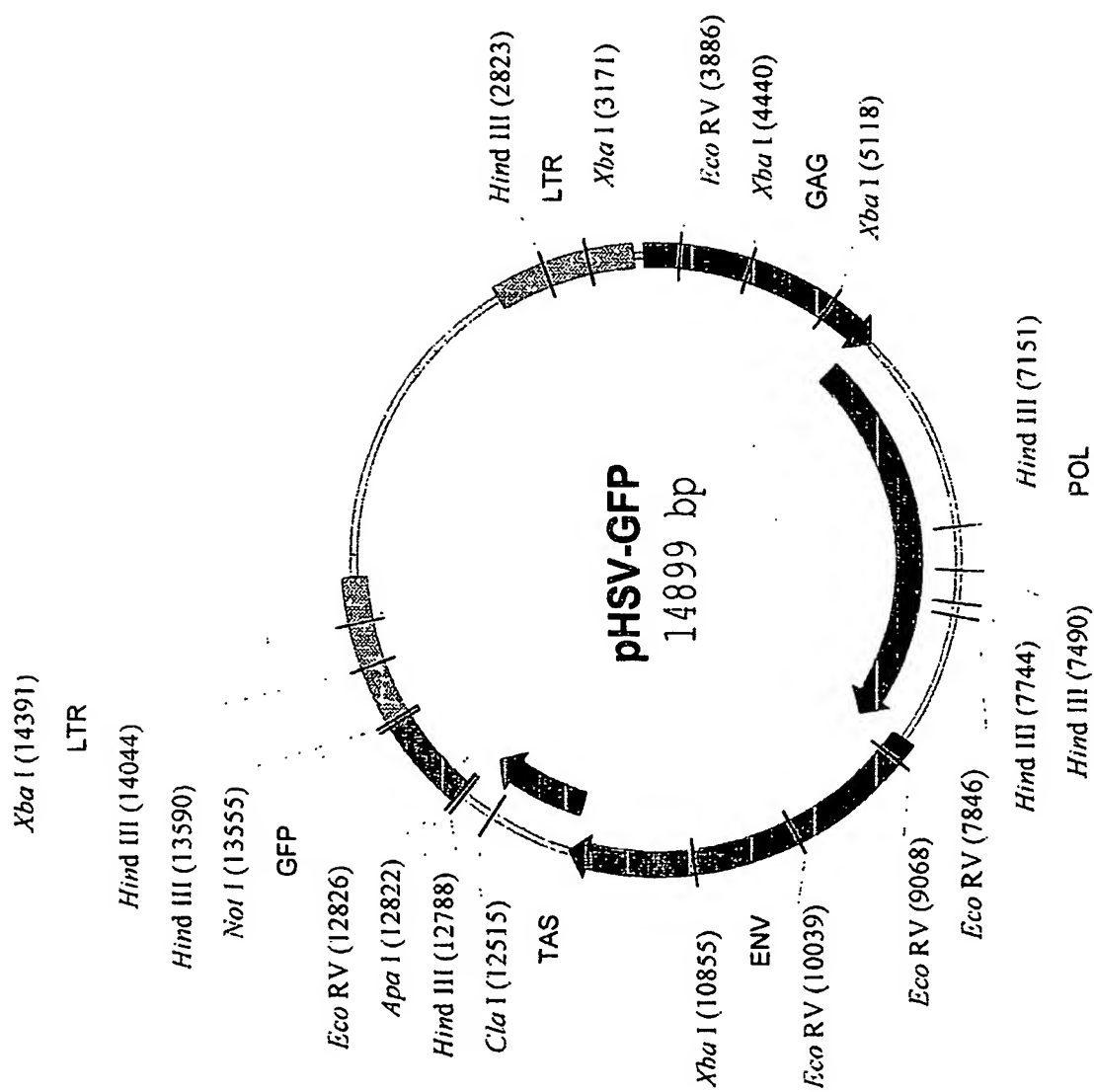


Fig. 1

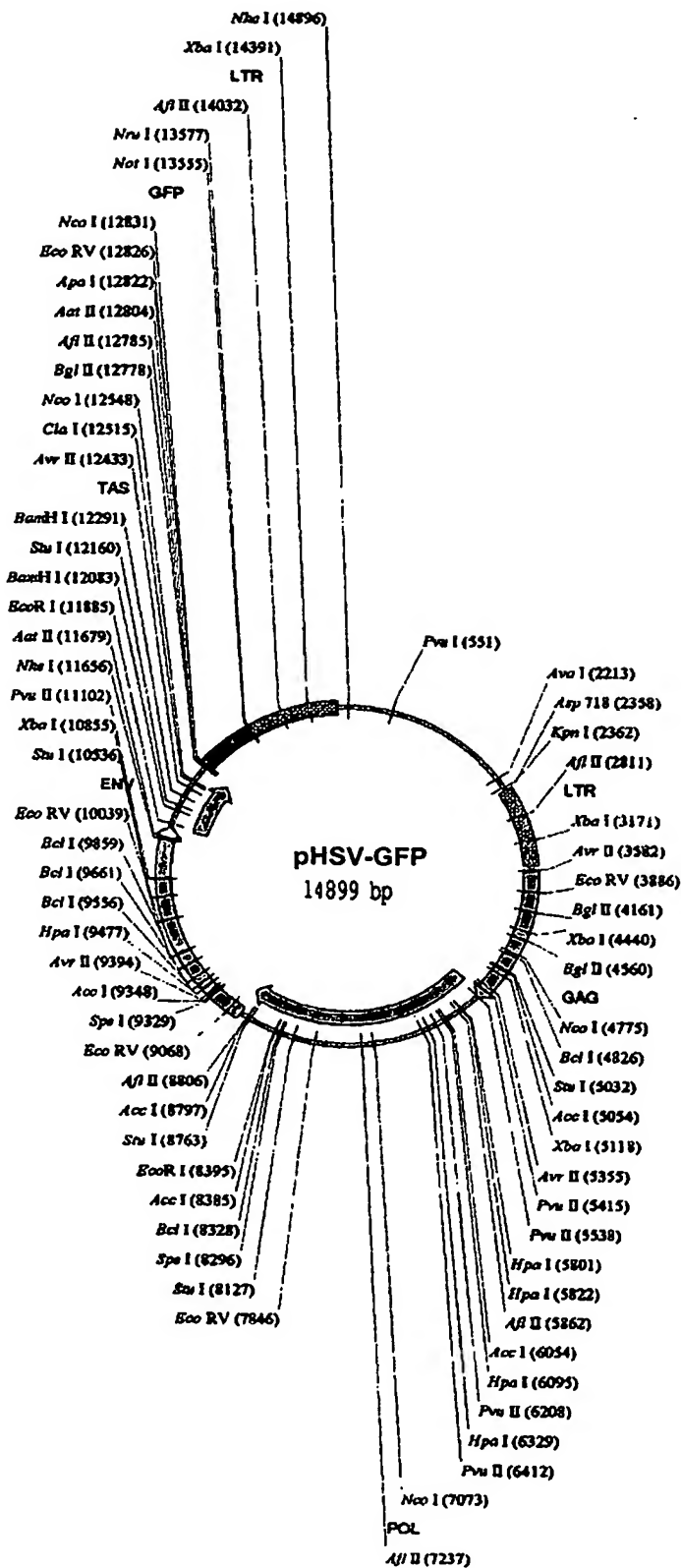


Fig. 2

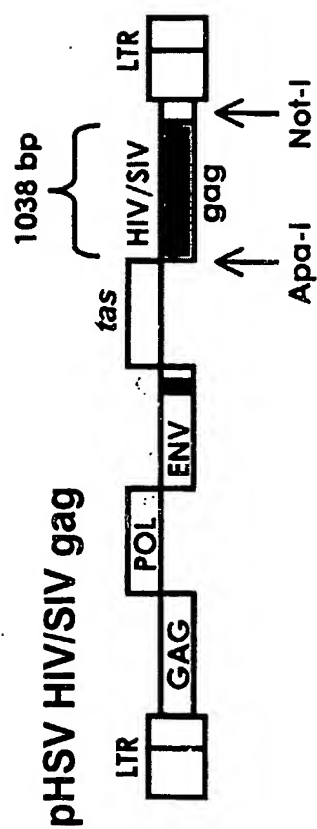


Fig. 3

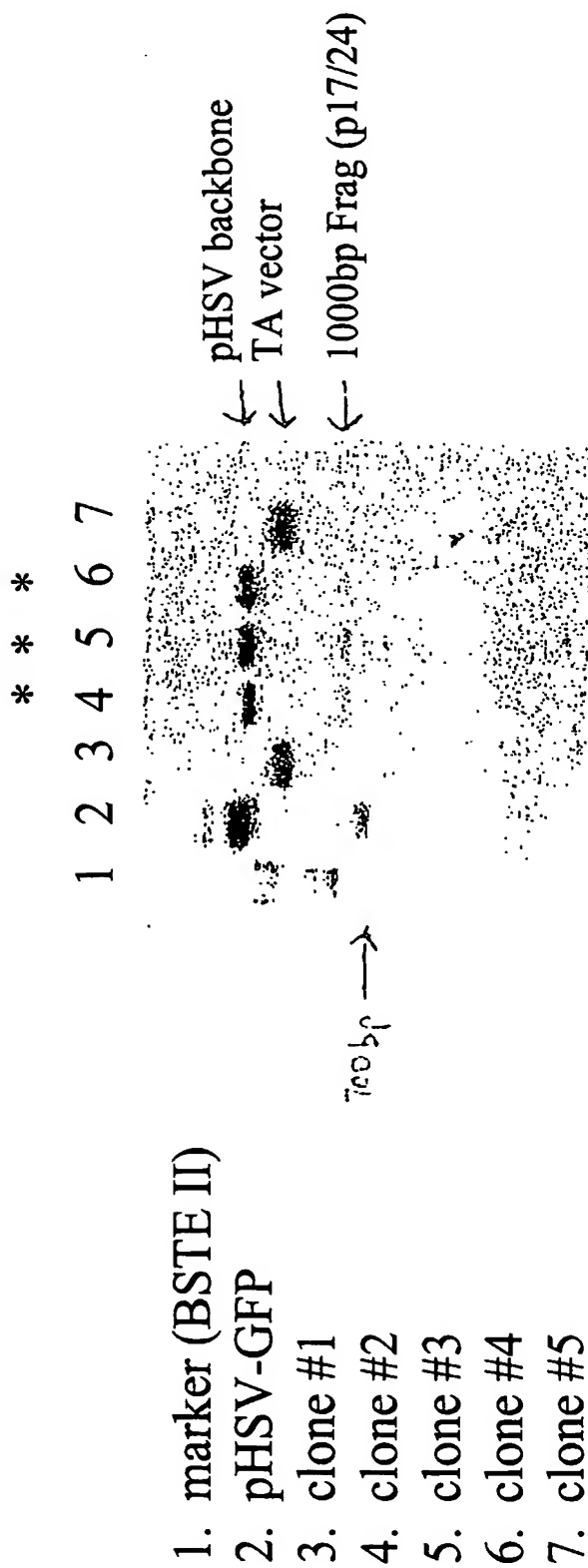


Fig. 4

gag expression post transfection

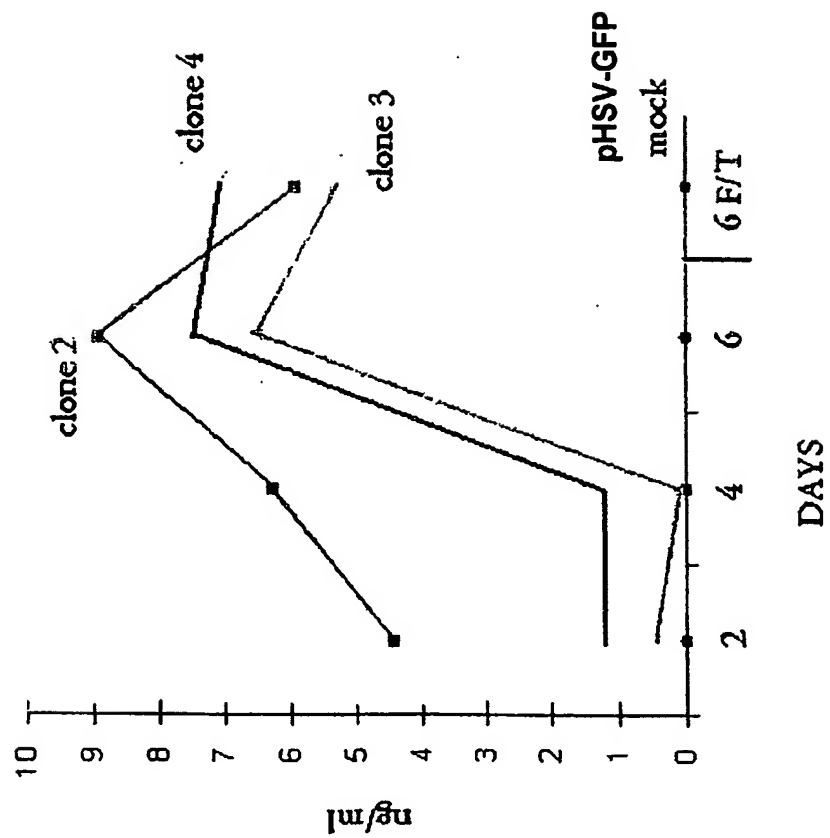
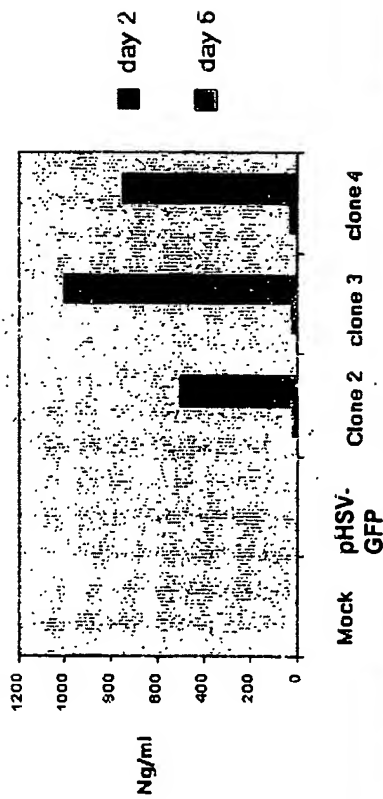


Fig. 5

p24 Expression of HFV containing gag sequences

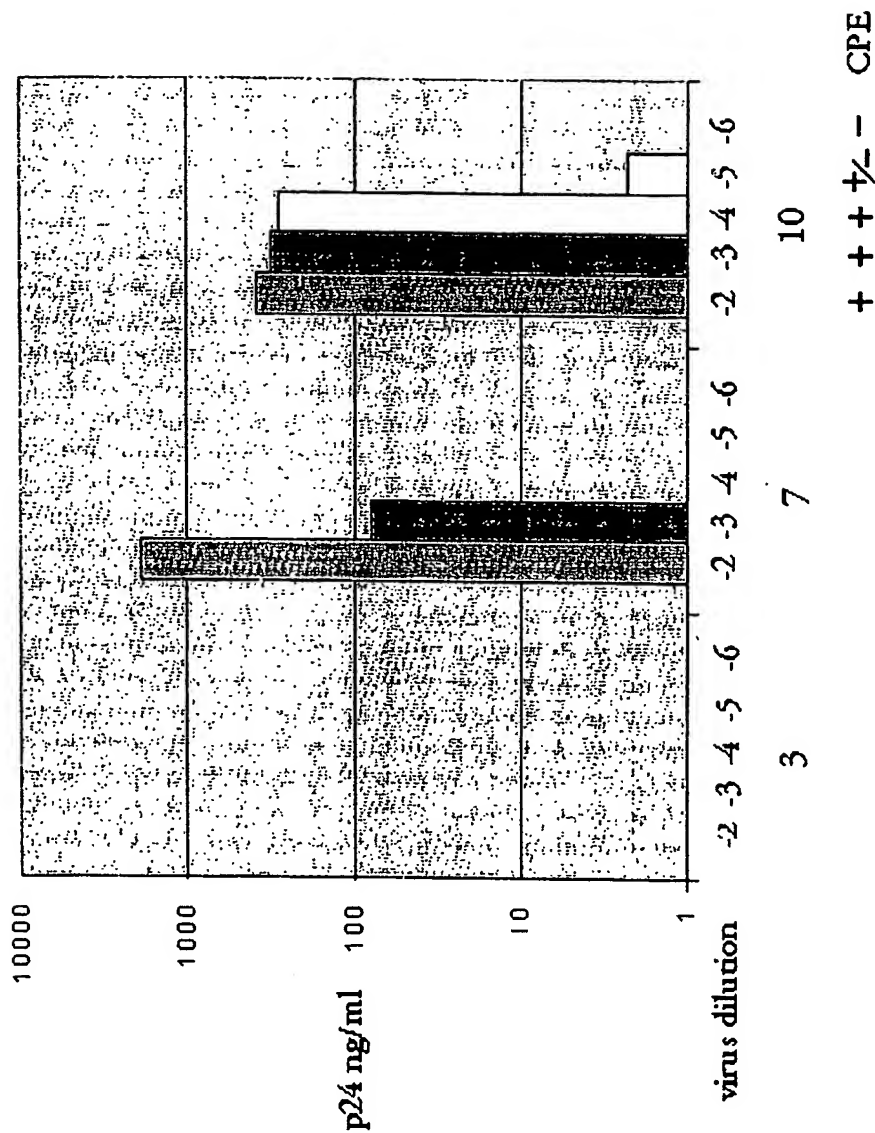


Cytopathic Effect

	Day 2	Day 5
Mock	-	-
pHSV-GFP	+/-	4+
clone 2	+/-	4+
clone 3	+/-	4+
clone 4	+/-	4+

Fig. 6

p24 Production of pHSV-HIV gag (2nd pass, clone 2)



DAYS POST INFECTION

Fig. 7

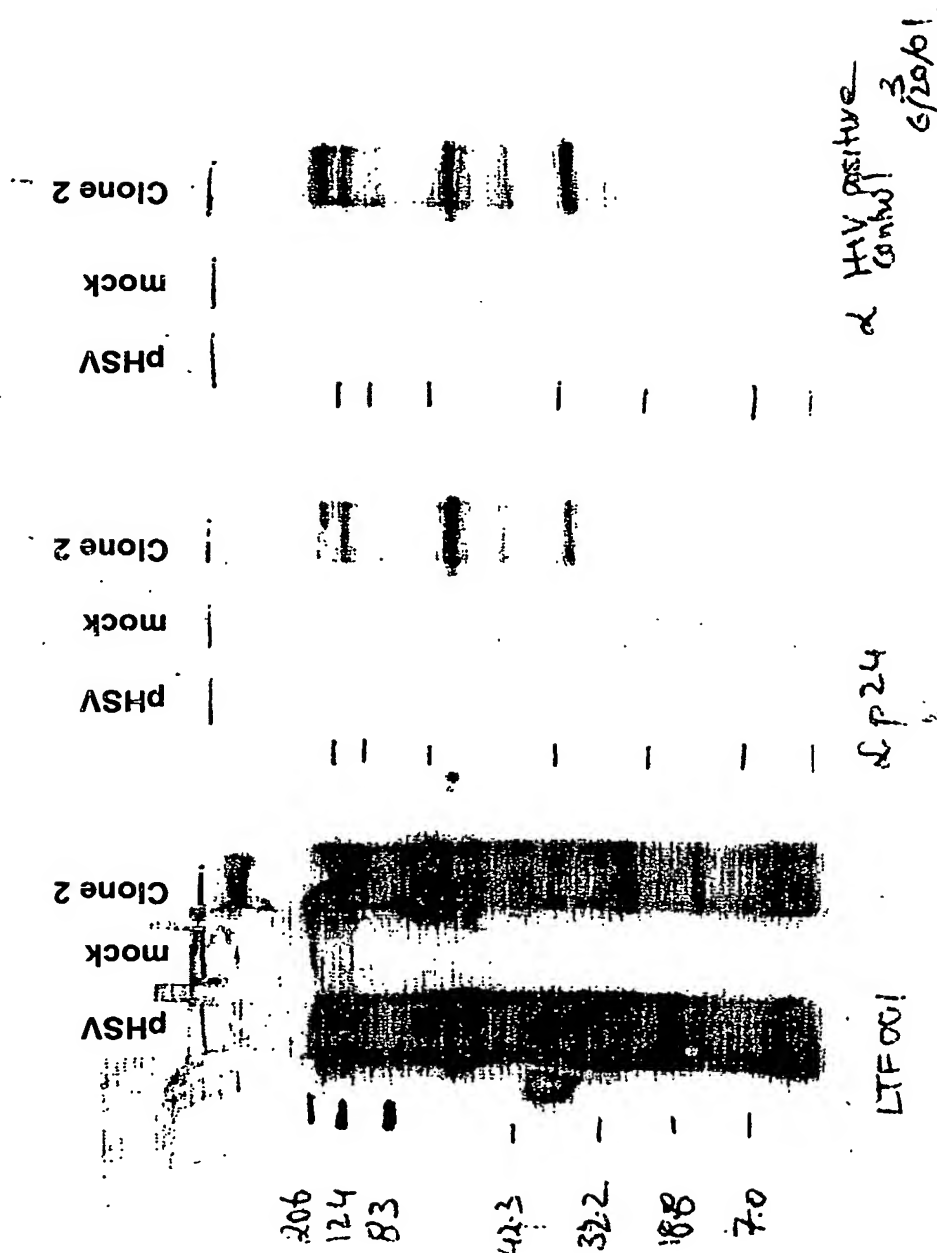


Fig. 8

SEQ. ID NO. I

```
1  CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT
   GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA
-----
51  TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCTTGATA
   AAGATTTATG TAAGTTTATA CATAGGCGAG TACTCTGTTA TTGGGACTAT
-----
101 AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC
   TTACGAAGTT ATTATAACTT TTTCTTCTC ATACTCATAA GTTGTAAGG
-----
151 GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCFTCC TGTTTTTGCT
   CACAGCGGGA ATAAGGGAAA AAACGCCGTA AAACGGAAGG ACAAAAACGA
-----
201 CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC
   GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG
-----
251 ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA
   TGCTCACCCA ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACCTC
-----
301 GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
   CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC
-----
351 CTATGTGGCG CGGTATTATC CCGTGTTGAC GCCGGGCAAG AGCAACTCGG
   GATACACCGC GCCATAATAG GGCACAACCTG CGGCCCCGTT CCGTTGAGCC
-----
401 TCGCCGCATA CACTATTCTC AGAATGACTT GGTGAGTAC TCACCAGTCA
   AGCGGCCTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT
-----
451 CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT
   GTCTTTTCGT AGAATGCCTA CCGTACTGTC ATTCTCTTAA TACGTCACGA
-----
501 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT
   CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA
-----
551 CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG
   GCCTCCTGGC TTCCTCGATT GGCAGAAAAA CGTGTGTGAC CCCCTAGTAC
-----
601 TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC
   ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG
-----
651 GACGAGCGTG ACACCACGAT GCCTGCAGCA ATGGCAACAA CGTTGCGCAA
   CTGCTCGCAC TGTGGTGCTA CGGACGTCGT TACC3TTGTT GCAACGCGTT
-----
701 ACTATTAACT GGCGAACCTAC TTA CTCTAGC TTCCCGSCAA CAATTAATAG
   TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC
-----
751 ACTGGATGGA GGCGGATAAA GTTGCAAGAC CACTTCTGCG CTCGGCCCTT
   TGACCTACCT CCGCCTATTT CAACGTCCTG GTGAAGACGC GAGCCGGGAA
-----
801 CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC
   GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG
-----
851 TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TCCATTCGGG AGGGCATAGC
   AGCGCCATAG TAACGTCGTG ACCCGGTCT AGGTAAGCCC TCCCGTATCG
-----
901 ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGGAAGCCC
   TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACCATTCGGG
-----
```

951 TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA
AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT

1001 ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT
TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA TTCGTAACCA

1051 AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAACTT
TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA

1101 CATTTTTAAT TTTAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT
GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC TATTAGAGTA

1151 GACCAAAATC CTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG
CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC

1201 TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC
ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAGA CGCGCATTAG

1251 TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC
ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG

1301 GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAAGTGGC TTCAGCAGAG
CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG AAGTCGTCTC

1351 CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC
GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG

1401 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT
AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG ATTAGGACAA

1451 ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGAAT
TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA

1501 CAAGACGATA GTTACCGGAT AAGCGCGCAGC GGTCGGGCTG AACGGGGGGT
GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC TTGCCCCCA

1551 TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA
AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT

1601 CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG
GGATGTCGCA CTCGATACTC TTTCGCGGTG CGAAGGGCTT CCTCTTTCC

1651 CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCAGCAGG
GCCTGTCCAT AGGCCATTCT CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC

1701 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTTG
CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC AGCCCAAAGC

1751 CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA
GGTGAGACT GAAGTCGCAG CTAAAAACAC TACGAGCAGT CCCCCGCCT

1801 GCCTATGGAA AAACGCCAGC AACCGGGCTT TTTTACGGTT CCTGGCCTTT
CGGATACCTT TTGCGGTCTG TTGCGCCGGA AAAATGCCAA GGACCGGAAA

1851 TGCTGGCCTT TTGCTCACAT GTTCTTTCTT GCGTTATCCC CTGATTCTGT
ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA

2851 CAATCAACAA AACAATGATG ATGTAATCAT AAGGAAGTAG TTTAAAATAG
GTTAGTTGTT TTGTTACTAC TACATTAGTA TTCCTTCATC AAATTTTATC

2901 GTTAATAAGT TTATTAGTTA TATAGAAAAT AATATAGGAT AAAGTATAAG
CAATTATTCA AATAATCAAT ATATCTTTTA TTATATCCTA TTTCATATTC

2951 GATTAAGGTA TGAGGTGTGT GGCTCAACAC GTAGGGTGAC AAGAAAATCT
CTAATTCAT ACTCCACACA CCGAGTTGTG CATCCCACTG TTCTTTTAGA

3001 ACTGTAATAG GACACAACAC CTCTAAAGTT GCCCGTGGGA AGGTGAAGTG
TGACATTATC CTGTGTTGTG GAGATTTCAA CGGGCACCTC TCCACTTCAC

3051 AGATCGAATC TTTCCTTAAC GCAGACAGCT TTTTATCCAC TAGGGATAAT
TCTAGCTTAG AAAGGAATTG CGTCTGTCTGA AAAATAGGTG ATCCCTATTA

3101 GTTTTAAGGA ATACTATAGT AATAGATTGA TAGTTTTAAC AATGATGGAA
CAAAATTCCT TATGATATCA TTATCTAACT ATCAAAATTG TTACTACCTT

3151 ATAGTATATA AGGATAGTTT CTAGATTGTA CGGGAGCTCT TCACTACTCG
TATCATATAT TCCTATCAAA GATCTAACAT GCCCTCGAGA AGTGATGAGC

3201 CTGCGTCGAG AGTGTCAGAG ACTCTCCAGG TTTGGTAAGA AATATTTTAT
GACGCAGCTC TCACATGCTC TGAGAGGTCC AAACCATTCT TTATAAATA

3251 ATTGTTATAA TGTTACTATG ATCCATTAAC ACTCTGCTTA TAGATTGTAA
TAACAATATT ACAATGATAC TAGGTAATTG TGAGACGAAT ATCTAACATT

3301 GGGTGATTGC AATGCTTTCT GCATAAACT TTGGTTTTCT TGTTAATCAA
CCCACTAACG TTACGAAAGA CGTATTTTGA AACCAAAAGA ACAATTAGTT

3351 TAAACCGACT TGATTGAGTA ACCTACTCAT ATATTATTGT CTCTTTTATA
ATTTGGCTGA ACTAAGCTCT TGGATGAGTA TATAATAACA GAGAAAATAT

3401 CTTTATTAAAG TAAAAGGATT TGTATATTAG CTTTGCTAAG GGAGACATCT
GAAATAATTG ATTTTCCTAA ACATATAATC GGAACGATTC CCTCTGTAGA

3451 AGTGATATAA GTGTGAACCT CACTTATCTT AAATGATGTA ACTCCTTAGG
TCACTATATT CACACTTGAT GTGAATAGAA TTTACTACAT TGAGGAATCC

3501 ATAATCAATA TACAAAATTC CATGACAATT GGCGCCCAAC GTGGGGCTCG
TATTAGTTAT ATGTTTTAAG GTACTGTTAA CCGCGGGTTG CACCCCGAGC

3551 AATATAAGTC GGGTTTATTT GTAAATTATC CCTAGGGACC TCCGAGCATA
TTATAATCAG CCCAAATAAA CATTTAATAG GGATCCCTGG AGGCTCGTAT

3601 GCGGGAGGCA TATAAAAGCC AATAGACAAT GGCTTCAGGA AGTAATGTTG
CGCCCTCCGT ATATTTTCGG TTATCTGTAA CCGAAGTCCT TCATTACAAC

3651 AAGAATATGA ACTTGATGTT GAAGCTCTGG TTGTAATTTT AAGAGATAGA
TTCTTATACT TGAACACAA CTTTCGAGACC AACATTAAAA TTCTCTATCT

3701 AATATACCAA GAAATCCTTT ACATGGAGAA GTTATAGGTC TTCGCCTTAC
TTATATGGTT CTTTAGGAAA TGTACCTCTT CAATATCCAG AAGCGGAATG

3751 TGAAGGATGG TGGGGACAAA TTGAGAGATT TCAGATGGTA CGTCTAATAT
ACTTCCTACC ACCCCTGTTT AACTCTCTAA AGTCTACCAT GCAGATTATA

3801 TACAAGATGA TGATAATGAA CCTTTACAGA GACCTAGATA TGAGGTAATA
ATGTTCTACT ACTATTACTT GGAAATGTCT CTGGATCTAT ACTCCATTAT

3851 CAACGAGCTG TAAACCCTCA TACAATGTTT ATGATATCAG GACCATTAGC
GTTGCTCGAC ATTTGGGAGT ATGTTACAAA TACTATAGTC CTGGTAATCG

3901 TGAACCTCAA TTAGCCTTTC AGGATTTAGA TTTACCTGAA GGTCCATTGA
ACTTGAAGTT AATCGGAAAG TCCTAAATCT AAATGGACTT CCAGGTAAC

3951 GGTTTGGTCC ATTGGCAAAT GGACATTATG TTCAAGGAGA TCCTTATAGT
CCAAACCAGG TAACCGTTTA CCTGTAATAC AAGTTCCTCT AGGAATATCA

4001 AGTTCTTACA GACCAGTAAC AATGGCCGAA ACAGCCCAA TGACTAGAGA
TCAAGAATGT CTGGTCATTG TTACCGGCTT TGTGGGTTT ACTGATCTCT

4051 TGAACCTGAA GATGTTCTTA ATACTCAAAG TGAAATAGAA ATTCAAATGA
ACTTGACCTT CTACAAGAAT TATGAGTTTC ACTTTATCTT TAAGTTTACT

4101 TAAATTTATT GGAGTTGTAT GAAGTTGAAA CTAGAGCTCT TAGAAGACAA
ATTTAAATAA CCTCAACATA CTTCAACTTT GATCTCGAGA ATCTTCTGTT

4151 TTAGCTGAGA GATCTAGTAC AGGGCAAGGA GGAATATCCC CAGGAGCTCC
AATCGACTCT CTAGATCATG TCCCGTTCCT CTTTATAGGG GTCCCTCGAGG

4201 TCGTTCTCGA CCACCAGTAA GCAGCTTCTC AGGGTTACCA AGTTTGCCCT
AGCAAGAGCT GGTGGTCATT CGTCGAAGAG TCCAATGGT TCAAACGGGA

4251 CTATACCTGG GATTCATCCC AGGGCACCTT CACCTCCAAG GGCAACTTCT
GATATGGACC CTAAGTAGGG TCCCGTGGAA GTGGAGGTTT CCGTTGAAGA

4301 ACTCCCGGAA ATATTCCTTG GAGTTTAGGA GATGATAGCC CACCTTCATC
TGAGGGCCTT TATAAGGAAC CTCAAATCCT CTACTATCGG GTGGAAGTAG

4351 TAGTTTTCTT GGACCCTCTC AACCTCGTGT TTCTTTCCAT CCGGGAAATC
ATCAAAGGA CCTGGGAGAG TTGGAGCACA AAGAAAGGTA GGCCCTTTAG

4401 CTTTTGTTGA AGAAGAAGGT CATAGACCTA GATCCAGTC TAGAGAAAGG
GAAAACAACT TCTTCTTCCA GTATCTGGAT CTAGGGTCAG ATCTCTTTCC

4451 AGAAGAGAAA TTCTTCTGCT TCCTGTACCG TCAGCACCTC CTATGATTCA
TCTTCTCTTT AAGAAGGACG AGGACATGGC AGTCGTGGAG GATACTAAGT

4501 GTATATACCA GTACCACTCT CACCACCGAT TGGCACGGTT ATACCTATTC
CATATATGGT CATGGTGGAG GTGGTGGCTA ACCGTGCCAA TATGGATAAG

4551 ASCATATCAG ATCTGTAACCT GGAGAGCCTC CTAGAAACCC AAGAGAAATA
TCGTATAGTC TAGACATTGA CCTCTCGGAG GATCTTTGGG TTCTCTTTAT

4601 CCAATTTGGC TAGGACGAAA TGCTCCTGCT ATAGATGGAG TGTTCCCTGT
GGTTAAACCG ATCCTGCTTT ACGAGGACGA TATCTACCTC ACAAGGGACA

4651 TACAACACCG GATCTAAGAT GCAGAATAAT TAATGCTATA CTAGGAGGAA
ATGTTGTGGC CTAGATTCTA CGTCTTATTA ATTACGATAT GATCCTCCTT

4701 ATATTGGGCT ATCATTAACC CCTGGAGACT GTTTAACATG GGAATCAGCA
TATAACCCGA TAGTAATTGG GGACCTCTGA CAAATTGTAC CCTGAGTCGT

4751 GTAGCCACCT TATTTATTAG AACCCATGGA ACTTTTCCAA TGCATCAGCT
CATCGGTGGA ATAAATAATC TTGGGTACCT TGAAAAGGTT ACGTAGTCGA

4801 TGGAAATGTA ATAAAAGGCA TAGTTGATCA AGAAGGAGTG GCAACAGCAT
ACCTTTACAT TATTTTCCGT ATCAACTAGT TCTTCCTCAC CGTTGTCGTA

4851 ATACTTTGGG AATGATGCTT TCTGGACAAA ATTATCAATT AGTTTCTGGA
TATGAAACCC TTACTACGAA AGACCTGTTT TAATAGTTAA TCAAAGACCT

4901 ATAATTAGAG GATATTTGCC TGGACAAGCT GTAGTAACTG CATTACAACA
TATTAATCTC CTATAAACGG ACCTGTTCTGA CATCATTGAC GTAATGTTGT

4951 GCGTTTACAG CAAGAAATAG ATAATCAAAC AAGAGCAGAG ACTTTTATTC
CGCAAATCTG GTTCTTTATC TATTAGTTTG TTCTCGTCTC TGAAAATAAG

5001 AACATCTAAA TGCTGTATAT GAAATTTTAG GCCTTAATGC CAGAGGACAA
TTGTAGATTT ACGACATATA CTTTAAAATC CGGAATTACG GTCTCCTGTT

5051 AGTATACGTG CTTCACTGAC TCCTCAACCC CGACCATCCA GAGGTAGAGG
TCATATGCAC GAAGTCACTG AGGAGTTGGG GCTGGTAGGT CTCCATCTCC

5101 TCGAGGTCAA AATACTTCTA GACCTCTCTA AGGACCAGCT AATAGCGGGC
AGCTCCAGTT TTATGAAGAT CTGGGAGAGT TCCTGGTCTG TTATCGCCCC

5151 GGGGACGACA GCGCCCTGCT TCTGGTCAAA GCAACAGAGG ATCTAGTACT
CCCCTGCTGT CGCGGGACGA AGACCAGTTT CGTTGTCTCC TAGATCATGA

5201 CAGAATCAAA ATCAAGATAA TTTAAATCAA GGAGGATATA ATCTTCGACC
GTCTTAGTTT TAGTTCTATT AAATTTAGTT CCTCCTATAT TAGAAGCTGG

5251 CCGTACTTAC CAACCTCAAA GGTACGGAGG AGGACSTGGA CGAAGATGGA
GGCATGAATG GTTGGAGTTT CCATGCCTCC TCCTGCACCT GCTTCTACCT

5301 ACGATAATAC TAACAATCAA GAGTCCAGAC CATCAGATCA AGGTTCTCAA
TGCTATTATG ATTGTTAGTT CTCAGGTCTG GTAGTCTAGT TCCAAGAGTT

5351 ACTCCTAGGC CAAATCAAGC AGGCTCTGGG GTGCGTGGCA ATCAGTCACA
TGAGGATCCG GTTAGTTCTG TCCGAGACCC CACGCACCGT TAGTCAGTGT

5401 AACTCCCAGA CCAGCTGCTG GTCGCGGAGG AAGAGGTAAC CACAAACGAA
TTGAGGCTCT GGTGACGAC CAGCGCCTCC TTCTCCATTG GTGTTGGCTT

5451 ACCAACGATC ATCCGGTGCT GGTGACTCAC GCGCTGTCAA TACCGTGACA
TGGTTGCTAG TAGGCCACGA CCACTGAGTG CGCGACAGTT ATGGCACTGT

5501 CAGAGTGCCA CGTCCTCCAC AGATGAATCC TCTTCAGCTG TTACAGCCGC
GTCTCACGGT GCAGGAGGTG TCTACTTAGG AGAAGTCGAC AATGTCGGCG

5551 TTCCGGCGGA GATCAAAGGG ACTAAATTGT TAGCCCACTG GGATTCAGGG
AAGGCCGCCT CTAGTTTCCC TGATTTAACA ATCGGGTGAC CCTAAGTCCC

5601 GCAACAATAA CTGTATTCC TGAAAGTTTT TTAGAAGATG AACAACTAT
CGTTGTTATT GAACATAAGG ACTTTCAAAA AATCTTCTAC TTGTTGGATA

5651 TAAAAAGACT TTAATAAAAA CAATTCATGG AGAAAAACAA CAAAATGTTT
ATTTTTCTGA AATTATTTTT GTTAAGTACC TCTTTTTGTT GTTTTACAA

5701 ATTATGTAAC CTTTAAAGTT AAAGGAAGAA AAGTGAAGC AGAAGTGATA
TAATACATTG GAAATTTCAA TTTCTTCTT TTCACCTTCG TCTTCACTAT

5751 GCTTCTCCTT ATGAGTATAT TTTGCTGTCTG CCAACAGATG TTCCTTGGTT
CGAAGAGGAA TACTCATATA AAACGACAGC GGTGTCTAC AAGGAACCAA

5801 AACACAGCAA CCACTTCAGT TAACAATTTT AGTTCCTCTT CAAGAATATC
TTGTGTCGTT GGTGAAGTCA ATTGTTAAAA TCAAGGAGAA GTTCTTATAG

5851 AAGAGAAAAT CTTAAGTAAG ACTGCTCTTC CAGAAGATCA AAAACAACAA
TTCTCTTTTA GAATTCATTG TGACGAGAAG GTCTTCTAGT TTTTGTGTT

5901 TTAAAAACCT TGTTTGTCAT GTATGACAAT CTATGGCAAC ATTGGGAAAA
AATTTTGGCA ACAAACAGTT CATACTGTGA GATACCGTTG TAACCCTTTT

5951 TCAAGTCGGG CATAGAAAAA TTAGGCCACA TAATATAGCA ACTGGTGATT
AGTTCAGCCC GTATCTTTTT AATCCGGTGT ATTATATCGT TGACCACTAA

6001 ATCCTCCTCG CCCTCAAAAA CAATATCCTA TTAATCCTAA GCCAAAGCCT
TAGGAGGAGC GGGAGTTTTT GTTATAGGAT AATTAGGATT CCGTTTCGGA

6051 AGTATACAAA TTGTAATAGA TGAATTATTG AAACAAGGGG TGTAAACGCC
TCATATGTTT AACATTATCT ACTGAATAAC TTTGTCCCTT ACAATTGCGG

6101 TCAAAATAGT ACAATGAATA CACCAGTGTA TCCTGTTCTT AAACCAGATG
AGTTTTATCA TGTTACTTAT GTGGTCACAT AGGACAAGGA TTTGGTCTAC

6151 GAAGGTGGAG AATGGTATTA GATTATAGAG AAGTAAATAA AACTATTCCA
CTTCCACCTC TTACCATAAT CTAATATCTC TTCATTTATT TTGATAAGGT

6201 TTAACAGCTG CCCAAAACCA AACTCTGCTT GGTATTTTAG CTACTATTGT
AATTGTCGAC GGGTTTTGGT TGTGAGACGA CCATAAAATC GATGATAACA

6251 TAGACAAAAA TATAAACTA CCTTAGATTT AGCTAATGGA TTTTGGGCTC
ATCTGTTTTT ATATTTTCAT GGAATCTAAA TCGATTACCT AAAACCCGAG

6301 ATCCTATTAC ACCAGAATCT TATTGGTTAA CAGCATTTAC CTGGCAAGGT
TAGGATAATG TGGTCTTAGA ATAACCAATT GTCGTAAATG GACCGTTCCA

6351 AAACAGTATT GTTGGACAAG TCTTCTCAA GGATTTTAA ATAGTCCAGC
TTTGTATATA CAACCTGTGC AGAAGGAGTT CCTAAAAAT TATCAGGTGC

6401 ATTGTTTACA GCTGATGTAG TAGATTTACT AAAAGAAATC CCTAATGTAC
TAACAAATGT CGACTACATC ATCTAAATGA TTTTCTTTAG GGATTACATG

6451 AAGTGTATGT TGATGATATA TATTTAAGCC ATGATGATCC TAAAGAGCAT
TTCACATACA ACTACTATAT ATAAATTCGG TACTACTAGG ATTTCTCGTA

6501 GTTCAACAAT TAGAAAAAGT GTTTCAAATT TTACTACAGG CAGGATATGT
CAAGTTGTTA ATCTTTTTCA CAAAGTTTAA AATGATGTCC GTCCTATACA

6551 AGTATCTTTG AAAAAATCAG AAATTGGTCA AAAAAGTGTG GAATTTTATG
TCATAGAAAC TTTTGTAGTC TTTAACCAGT TTTTGTACAT CTTAAAAATC

6601 GATTTAATAT TACTAAAGAA GGTCGTGGCC TAACAGACAC TTTTAAACA
CTAAATTATA ATGATTTCTT CCAGCACCGG ATTGTCTGTG AAAATTTTGT

6651 AAACGTGTTAA ATATTACTCC TCCAAAAGAC TTAAAGCAAT TACAAAGCAT
TTTGACAATT TATAATGAGG AGGTTTTCTG AATTTCTGTTA ATGTTTCGTA

6701 ATTAGGATTG TTAAATTTTG CTAGAAATTT TATACCTAAT TTTGCTGAAC
TAATCCTAAC AATTTAAAAC GATCTTTAAA ATATGGATTA AAACGACTTG

6751 TGGTACAACC ATTATACAAT TTAATAGCCT CAGCAAAAGG CAAATATATT
ACCATGTTGG TAATATGTTA AATTATCGGA GTCGTTTTCC GTTTATATAA

6801 GAGTGGTCTG AAGAAAATAC TAAACAATTA AATATGGTAA TAGAAGCATT
CTCACCAGAC TTCTTTTATG ATTTGTTAAT TTATACCATT ATCTTCGTAA

6851 AAACACTGCC TCTAATTTAG AAGAAAGGTT ACCAGAACAG AGACTGGTAA
TTTGTGACGG AGATTAAATC TTCTTTCCAA TGGTCTTGTC TCTGACCATT

6901 TTAAAGTCAA TACTTCTCCA TCAGCAGGAT ATGTAAGATA TTATAATGAG
AATTTCAAGT ATGAAGAGGT AGTCCTCCTA TACATTCTAT AATATTACTC

6951 ACTGGTAAAA AGCCTATTAT GTACCTAAAT TATGTGTTTT CCAAAGCAGA
TGACCATTTT TCGGATAATA CATGGATTTA ATACACAAAA GGTTTCGTCT

7001 ATTAAAATTT TCTATGTTAG AAAAATCTATT AACTACAATG CACAAAGCCT
TAATTTTAAA AGATACAATC TTTTGTGATAA TTGATGTTAC GTGTTTCGGA

7051 TAATTAAGGC TATGGATTGG GCCATGGGAC AAGAAATATT AGTTTATAGT
ATTAATTCCG ATACCTAAAC CGGTACCCTG TTCTTTATAA TCAAATATCA

7101 CCCATTGTAT CTATGACTAA AATACAAAAA ACTCCACTAC CAGAAAGAAA
GGGTAACATA GATACTGATT TTATGTTTTT TGAGGTGATG GTCTTTCTTT

7151 AGCTTTACCC ATCAGATGGA TAACATGGAT GACTTATTTA GAAGATCCAA
TCGAAATGGG TAATCTACCT ATTGTACCTA CTGAATAAAT CTTCTAGGTT

7201 GAATCCAATT TCATTATGAT AAAACCTTAC CAGAACTTAA GCATATTCCA
CTTAGGTTAA AGTAATACTA TTTTGAATG GTCTTGAATT CGTATAAGGT

7251 GATGTATATA CATCTAGTCA GTCTCCTGTT AAACATCCTT CTCAATATGA
CTACATATAT GTAGATCAGT CAGAGGACAA TTTGTAGGAA GAGTTATACT

7301 AGGAGTGTTC TATACTGATG GCTCGGCCAT CAAAAGTCCT GATCCTACAA
TCCTCACAAA ATATGACTAC CGAGCCGGTA GTTTTCAGGA CTAGGATGTT

7351 AAAGCAATAA TGCTGGCATG GGAATAGTAC ATGCCACATA CAAACCTGAA
TTTCGTATT ACGACCCTAC CCTTATCATG TACGGTGTAT GTTTGGACTT

7401 TATCAAGTTT TGAATCAATG GTCAATACCA CTAGGTAATC ATACTGCTCA
ATAGTTCAAA ACTTAGTTAC CAGTTATGGT GATCCATTAG TATGACGAGT

7451 GATGGCTGAA ATAGCTGCAG TTGAATTTGC CTGTAAAAA GCTTTAAAAA
CTACCGACTT TATCGACGTC AACTTAAACG GACATTTTTT CGAAATTTTT

7501 TACCTGGTCC TGTATTAGTT ATAACGTATA GTTTCTATGT AGCAGAAAGT
ATGGACCAGG ACATAATCAA TATTGACTAT CAAAGATACA TCGTCTTTCA

7551 GCTAATAAAG AATTACCATA CTGGAAATCT AATGGGTTTG TTAATAATAA
CGATTATTTT TTAATGGTAT GACCTTTAGA TTACCCAAAC AATTATTATT

7601 GAAAAAGCCT CTAAACATA TCTCAAATG GAAATCTATT GCTGAGTGTT
CTTTTTCGGA GAATTTGTAT AGAGGTTTAC CTTTAGATAA CGACTCACAA

7651 TATCTATGAA ACCAGACATT ACTATTCAAC ATGAAAAAGG CATCAGCCTA
ATAGATACTT TGGTCTGTAA TGATAAGTTG TACTTTTTCC GTAGTCGGAT

7701 CAAATACCAG TATTCATACT GAAAGGCAAT GCCCTAGCAG ATAAGCTTGC
GTTTATGGTC ATAAGTATGA CTTTCCGTTA CGGGATCGTC TATTCGAACG

7751 CACCCAAGGA AGTTATGTGG TTAATTGTAA TACCAAAAAA CCAAACCTGG
GTGGGTTTCT TCAATACACC AATTAACATT ATGGTTTTTTT GGTTTGGACC

7801 ATGCAGAGTT GGATCAATTA TTACAGGGTC ATTATATAAA AGGATATCCC
TACGTCTCAA CCTAGTTAAT AATGTCCCAG TAATATATTT TCCTATAGGG

7851 AAACAATATA CATATTTTTT AGAAGATGGC AAAGTAAAAG TTTCCAGACC
TTTGTTATAT GTATAAAAAA TCTTCTACCG TTTCATTTTC AAAGGTCTGG

7901 TGAAGGGGTT AAAATTATTC CCCCTCAGTC AGACAGACAA AAAATTGTGC
ACTTCCCCAA TTTTAATAAG GGGGAGTCAG TCTGTCTGTT TTTTAACACG

7951 TTCAAGCCCA CAATTTGGCT CACACCGGAC GTGAAGCCAC TCTTTTAAAA
AAGTTCGGGT GTTAAACCGA GTGTGGCCTG CACTTCGGTG AGAAAATTTT

8001 ATTGCCAACC TTTATTGGTG GCCAAATATG AGAAAGGATG TGGTTAAACA
TAACGGTTGG AAATAACCAC CGGTTTATAC TCTTCTCTAC ACCAATTTGT

8051 ACTAGGACGC TGTCAACAGT GTTTAATCAC AAATGCTTCC AACAAAGCCT
TGATCCTGCG ACAGTTGTCA CAAATTAGTG TTTACGAAGG TTGTTTCGGA

8101 CTGGTCCTAT TCTAAGACCA GATAGGCCTC AAAAACCTTT TGATAAATTC
GACCAGGATA AGATTCTGGT CTATCCGGAG TTTTGGGAAA ACTATTTAAG

8151 TTTATTGACT ATATTGGACC TTTGCCACCT TCACAGGGAT ACCTATATGT
AAATAACTGA TATAACCTGG AAACGGTGGA AGTGTCCCTA TGGATATACA

8201 ATTAGTAGTT GTTGATGGAA TGACAGGATT CACTTGGTTA TACCCCACTA
TAATCATCAA CAACTACCTT ACTGTCCTAA GTGAACCAAT ATGGGGTGAT

8251 AGGCTCCTTC TACTAGCGCA ACTGTAAAT CTCTCAATGT ACTCACTAGT
TCCGAGGAAG ATGATCGCGT TGACAATTTA GAGAGTTACA TGAGTGATCA

8301 ATTGCAATTC CAAAGGTGAT TCACTCTGAT CAAGGTGCAG CATTCACTTC
TAACGTTAAG GTTTCCTACTA AGTGAGACTA GTTCCACGTC GTAAGTGAAG

8351 TTCAACCTTT GCTGAATGGG CAAAGGAAAG AGGTATACAT TTGGAATTCA
AAGTTGGAAA CGACTTACCC GTTTCCTTTC TCCATATGTA AACCTTAAGT

8401 GTACTCCTTA TCACCCCAA AGTGGTAGTA AGGTGGAAAG GAAAAATAGT
CATGAGGAAT AGTGGGGGTT TCACCATCAT TCCACCTTTC CTTTTTATCA

8451 GATATAAAAC GACTTTTAACT TAACTGCTA GTAGGAAGAC CCACAAAGTG
CTATATTTTG CTGAAAATTG ATTTGACGAT CATCCTTCTG GGTGTTTCAC

8501 GTATGACCTA TTGCCTGTTG TACAACCTGC TTTAAACAAC ACCTATAGCC
CATACTGGAT AACGGACAAC ATGTTGAACG AAATTTGTTG TGGATATCGG

8551 CTGTATTAAA ATATACTCCA CATCAACTCT TATTTGGTAT AGATTCAAAT
GACATAATTT TATATGAGGT GTAGTTGAGA ATAAACCATA TCTAAGTTTA

8601 ACTCCATTTG CAAATCAAGA TACACTTGAC TTGACCAGAG AAGAAGAAGT
TGAGGTAAAC GTTTAGTTCT ATGTGAACTG AACTGGTCTC TTCTTCTTGA

8651 TTCTCTTTTA CAGGAAATTC GTACTTCTTT ATACCATCCA TCCACCCCTC
AAGAGAAAAT GTCCTTTAAG CATGAAGAAA TATGGTAGGT AGGTGGGGAG

8701 CAGCCTCCTC TCGTTCCTGG TCTCCTGTTG TTGGCCAATT GGTCCAGGAG
GTCGGAGGAG AGCAAGGACC AGAGGACAAC AACCGGTTAA CCAGGTCCTC

8751 AGGGTGGCTA GGCCTGCTTC TTTGAGACCT CGTTGGCATA AACCGTCTAC
TCCCACCGAT CCGGACGAAG AAACCTCTGGA GCAACCGTAT TTGGCAGATG

8801 TGTACTTAAG GTGTTGAATC CAAGGACTGT TGTTATTTTG GACCATCTTG
ACATGAATTC CACAACCTAG GTTCCTGACA ACAATAAAAC CTGGTAGAAC

8851 GCAACAACAG AACTGTAAGT ATAGATAATT TAAACCTAC TTCTCATCAG
CGTTGTTGTC TTGACATTCA TATCTATTAA ATTTTGGATG AAGAGTAGTC

8901 AATGGCACCA CCAATGACAC TGCAACAATG GATCATTTGG AAAAAATGA
TTACCGTGGT GGTACTGTG ACGTTGTTAC CTAGTAAACC TTTTTTACT

8951 ATAAAGCGCA TGAGGCACTT CAAAATACAA CAACTGTGAC TGAACAGCAG
TATTTGCGGT ACTCCGTGAA GTTTTATGTT GTTGACACTG ACTTGTCGTC

9001 AAGGAACAAA TTATACTGGA CATTCAAAAT GAAGAAGTAC AACCAACTAG
TTCCTTGTTT AATATGACCT GTAAGTTTTA CTTCTTCATG TTGGTTGATC

9051 GAGAGATAAA TTTAGATATC TGCTTTATAC TTGTTGTGCT ACTAGCTCAA
CTCTCTATTT AAATCTATAG ACGAAATATG AACAACACGA TGATCGAGTT

9101 GAGTATTGGC CTGGATGTTT TTAGTTTGTA TATTGTTAAT CATTGTTTTG
CTCATAACCG GACCTACAAA AATCAAACAT ATAACAATTA GTAACAAAAC

9151 GTTTCATGCT TTGTGACTAT ATCCAGAATA CAATGGAATA AGGATATTCA
CAAAGTACGA AACACTGATA TAGGTCTTAT GTTACCTTAT TCCTATAAGT

9201 GGTATTAGGA CCTGTAATAG ACTGGAATGT TACTCAAAGA GCTGTTTATC
CCATAATCCT GGACATTATC TGACCTTACA ATGAGTTTCT CGACAAATAG

9251 AACCTTACA GACTAGAAGG ATTCACGTT CCCTTAGAAT GCAGCATCCT
TTGGGAATGT CTGATCTTCC TAACGTGCAA GGAATCTTA CGTCGTAGGA

9301 GTTCCAAAAT ATGTGGAGGT AAATATGACT AGTATTCCAC AAGGTGTATA
CAAGGTTTTA TACACCTCCA TTTATACTGA TCATAAGGTG TTCCACATAT

9351 CTATGAACCC CATCCGGAAC CCATAGTGGT GAAGGAGAGG GTCCTAGGTC
GATACTGGG GTAGGCCTTG GGTATCACCA CTTCTCTCTC CAGGATCCAG

9401 TTTCTCAAAT TCTGATGATT AATTCAGAAA ACATTGCTAA TAATGCTAAT
AAAGAGTTTA AGACTACTAA TTAAGTCTTT TGTAACGATT ATTACGATTA

9451 TTGACACAAG AAGTAAAGAA GTTGTTAACT GAAATGGTTA ATGAAGAAAT
AACTGTGTTT TTCATTTCTT CAACAATTGA CTTTACCAAT TACTTCTTTA

9501 GCAAAGTTTG TCAGATGTAA TGATTGACTT TGAAATTCCT TTAGGAGACC
CGTTTCAAAC AGTCTACATT ACTAACTGAA ACTTTAAGGA AATCCTCTGG

9551 CTCGTGATCA AGAACAATAT ATACATAGAA AATGCTATCA AGAATTTGCA
GAGCACTAGT TCTTGTTATA TATGTATCTT TTACGATAGT TCTTAAACGT

9601 AATTGTTATT TAGTAAAATA TAAAGAACCC AAACCGTGGC CTAAGGAGGG
TTAACAATAA ATCATTTTAT ATTTCTTGGG TTTGGCACCG GATTCTCTCC

9651 CCTTATAGCT GATCAATGCC CATTACCAGG TTACCATGCT GGATTAACCT
GGAATATCGA CTAGTTACGG GTAATGGTCC AATGGTACGA CCTAATTGGA

9701 ATAATAGACA GTCTATTTGG GATTACTATA TTAAAGTGGA GAGTATTAGA
TATTATCTGT CAGATAAACC CTAATGATAT AATTTCACCT CTCATAATCT

9751 CCTGCAAATT GGACAACAAA GAGTAAATAT GGACAAGCTA GACTAGGAAG
GGACGTTTAA CCTGTTGTTT CTCATTTATA CCTGTTCGAT CTGATCCTTC

9801 TTTTATATT CCTAGCAGCC TGAGACAAAT CAATGTTAGT CATGTACTAT
AAAAATATAA GGATCGTCGG ACTCTGTTTA GTTACAATCA GTACATGATA

9851 TCTGTAGTGA TCAATTATAT TCTAAATGGT ATAATATAGA AAATACCATA
AGACATCACT AGTTAATATA AGATTTACCA TATTATATCT TTTATGGTAT

9901 GAACAAACG AGCGGTTTCT GCTTAATAAA CTAAATAACC TTACATCTGU
CTTGTTTTGC TCGCCAAAGA CGAATTATTT GATTTATTGG AATGTAGACC

9951 AACCTCAGTA TTGAAGAAAA GAGCTCTTCC GAAGGATTGG AGTTCTCAAG
TTGGAGTCAT AACTTCTTTT CTCGAGAAGG CTTCCCTAACC TCAAGAGTTC

10001 GTAAAAATGC TCTGTTTAGA GAAATCAATG TGTTAGATAT CTGCAGTAAA
CATTTTTACG AGACAAATCT CTTTAGTTAC ACAATCTATA GACGTCATTT

10051 CCTGAATCTG TAATACTATT GAATACTTCA TACTATTCTT TCTCTTTATG
GGACTTAGAC ATTATGATAA CTTATGAAGT ATGATAAGGA AGAGAAATAC

10101 GGAAGGAGAT TGTAATTTTA CTAAAGATAT GATTTCTCAG TTGGTTCCAG
CCTTCTCTA ACATTAAAAAT GATTTCTATA CTAAAGAGTC AACCAAGGTC

10151 AATGTGATGG ATTTTATAAC AATTCTAAGT GGATGCATAT GCATCCATAT
TTACACTACC TAAATATTG TTAAGATTCA CCTACGTATA CGTAGGTATA

10201 GCTTGTAGAT TCTGGAGAAG TAAGAAGAAT GAAAAAGAAG AAATAAATG
CGAACATCTA AGACCTCTTC ATTCTTCTTA CTTTTCTTC TTTGATTAC

10251 TAGAGATGGG GAAACTAAGA GATGTCTGTA TTATCCTTTA TGGGACAGTC
ATCTCTACCC CTTTGATTCT CTACAGACAT AATAGGAAAT ACCCTGTCAG

10301 CCGAATCTAC ATATGATTTT GGTTATTTAG CATACCAAAA GAATTTTCCT
GGCTTAGATG TATACTAAAA CCAATAAATC GTATGGTTTT CTTAAAAGGA

10351 TCCCCTATCT GTATAGAACA ACAGAAAATT AGAGATCAAG ATTATGAAGT
AGGGGATAGA CATATCTTGT TGTCTTTTAA TCTCTAGTTC TAATACTTCA

10401 CTATTCTTTG TATCAAGAAC GCAAATAGC TTCTAAAGCA TATGGAATTG
GATAAGAAAC ATAGTTCTTG CGTTTTATCG AAGATTCGT ATACCTTAAC

10451 ATACAGTTTT ATTCTCTCTA AAGAATTTTC TTAATTATAC AGGAACTCCT
TATGTCAAAA TAAGAGAGAT TTCTTAAAAG AATTAATATG TCCTTGAGGA

10501 GTAAATGAAA TGCCTAATGC AAGAGCTTTT GTAGGCCTAA TAGATCCCAA
CATTACTTTT ACGGATTACG TTCTCGAAAA CATCCGGATT ATCTAGGGTT

10551 GTTTCCTCCT TCCTATCCCA ATGTTACTAG GGAACATTAT ACTTCCTGTA
CAAAGGAGGA AGGATAGGGT TACAATGATC CTTTGTAAATA TGAAGGACAT

10601 ATAATAGGAA AAGAAGAAGT GTTGATAATA ACTATGCTAA GTTAAGGTCT
TATTATCCTT TTCTTCTTCA CAACTATTAT TGATACGATT CAATTCCAGA

10651 ATGGGGTATG CACTTACAGG AGCAGTGCAA ACCTTATCTC AAATATCAGA
TACCCCATAC GTGAATGTCC TCGTACGTT TGGAATAGAG TTTATAGTCT

10701 TATTAATGAT GAAAACCTTAC AGCAAGGAAT ATATTTATTA AGGGATCATG
ATAATTACTA CTTTGAATG TCGTTCCTTA TATAAATAAT TCCCTAGTAC

10751 TAATAACCTT AATGGAAGCT ACATTGCATG ATATATCTGT TATGGAAGGA
ATTATTGGAA TTACCTTCGA TGTAACGTAC TATATAGACA ATACCTTCCT

10801 ATGTTTGCTG TACAACATTT GCATACACAT TTGAATCATT TGAAGACAAT
TACAAACGAC ATGTTGTAAA CGTATGTGTA AACTTAGTAA ACTTCTGTTA

10851 GCTTCTAGAA AGAAGAATAG ACTGGACCTA TATGTCTAGT ACTTGGCTAC
CGAAGATCTT TCTTCTTATC TGACCTGGAT ATACAGATCA TGAACCGATG

10901 AACAACAATT ACAGAAATCT GATGATGAGA TGAAAGTAAT AAAGAGAATT
TTGTTGTAA TGTCTTTAGA CTACTACTCT ACTTTCATTA TTTCTCTTAA

10951 GCTAGAAGTT TGGTATATTA TGTAAACAA ACCCATAGTT CTCCACAGC
CGATCTTCAA ACCATATAAT ACAATTTGTT TGGGTATCAA GAGGGTGTGC

11001 TACAGCCTGG GAGATTGGAT TATATTATGA ATTGGTTATA CCTAAACATA
ATGTCGGACC CTCTAACCTA ATATAATACT TAACCAATAT GGATTGTAT

11051 TTTACTTGAA TAATTGGAAT GTTGTCATA TAGGTCACTT AGTTAAATCA
AAATGAACTT ATTAACCTTA CAACAGTTAT ATCCAGTGAA TCAATTTAGT

11101 GCTGGACAAT TGACTCATGT AACTATAGCT CATCCTTATG AAATAATCAA
CGACCTGTTA ACTCAGTACA TTGATATCGA GTAGGAATAC TTTATTAGTT

11151 TAAGGAATGT GTAGAGACTA TATATCTGCA TCTTGAGGAC TGCACAAGAC
ATTCTTACA CATCTCTGAT ATATAGACGT AGAACTCCTG ACGTGTTCGT

11201 AAGATTATGT CATATGTGAT GTGGTAAAGA TAGTGCAGCC TTGTGGCAAT
TTCTAATACA GTATACACTA CACCATTCT ATCACGTCGG AACACCGTTA

11251 AGCTCAGACA CGAGTGATTG TCCTGTCTGG GCTGAAGCTG TAAAAGAACC
TCGAGTCTGT GCTCACTAAC AGGACAGACC CGACTTCGAC ATTTTCTTGG

11301 ATTTGTGCAA GTCAATCCTC TGAAAAACGG AAGTTATCTG GTTTTGGCAA
TAAACACGTT CAGTTAGGAG ACTTTTGTCC TTCAATAGAC CAAAACCGTT

11351 GTTCCACAGA CTGTCAGATC CCACCATATG TTCCTAGCAT CGTGACTGTT
CAAGGTGTCT GACAGTCTAG GGTGTATAC AAGGATCGTA GCACTGACAA

```

11401  AATGAAACAA CGTCATGCTT TGGACTGGAC TTTAAAAGGC CACTGGTTGC
      TTACTTTGTT GCAGTACGAA ACCTGACCTG AAATTTTCCG GTGACCAACG
-----
11451  GGAAGAAAGA TTGAGCTTTG AGCCACGACT GCCAAATCTA CAACTAAGAT
      CCTTCTTTCT AACTCGAAAC TCGGTGCTGA CGGTTTAGAT GTTGATTCTA
-----
11501  TACCACATTT GGTGGAATT ATTGCAAAA TCAAAGGGAT AAAAATAGAA
      ATGGTGTAAG CCAACCTTAA TAACGTTTTT AGTTTCCCTA TTTTATCTT
-----
11551  GTCACATCCT CTGGAGAAAG TATAAAAGAG CAGATTGAAA GAGCAAAAGC
      CAGTGATAGG GACCTCTTTC ATATTTTCTC GTCTAACTTT CTCGTTTTTC
-----
11601  TGAGCTCCTT CGACTGGACA TTCACGAGGG AGATACTCCT GCCTGCATAC
      ACTCGAGGAA GCTGACCTGT AAGTGCTCCC TCTATGAGGA CGGACCTATG
-----
11651  AACAGCTAGC TGCAGCAACA AAGGACGTCT GGCCAGCAGC AGCTTCTGCT
      TTGTCGATCG ACGTCGTTGT TTCCTGCAGA CCGGTCGTCG TCGAAGACGA
-----
11701  CTACAAGGAA TTGGTAACCT TTTATCTGGG ACTGCCCAAG GAATATTTGG
      GATGTTCTTT AACCATTGAA AAATAGACCC TGACGGGTTC CTTATAAACC
-----
11751  AACTGCCTTT AGTCTCTTGG GATACTTAAA GCCTATCCTA ATAGGAGTAG
      TTGACGGAAA TCAGAGAACC CTATGAATTT CGGATAGGAT TATCCTCATC
-----
11801  GGGTCATTCT CTTGGTTATT CTTATATTTA AGATTGTATC ATGGATTCTT
      CCCAGTAAGA GAACCAATAA GAATATAAAT TCTAACATAG TACCTAAGGA
-----
11851  ACGAAAAAGA AGAATCAGTA GCCTCCACCT CTGGAATTCA AGACCTGCAG
      TGCTTTTTCT TCTTAGTCAT CGGAGGTGGA GACCTTAAGT TCTGGACGTC
-----
11901  ACTCTGAGTG AGCTTGTTGG TCCTGAAAAT GCCGGAGAGG GAGAGCTGAC
      TGAGACTCAC TCGAACAACC AGGACTTTTA CGGCCTCTCC CTCTCGACTG
-----
11951  TATTGCTGAG GAACCTGAAG AAAATCCTCG ACGCCCCAGA CGATATACTA
      ATAACGACTC CTTGGACTTC TTTTAGGAGC TCGGGGTCT GCTATATGAT
-----
12001  AAAGAGAAGT CAAATGTGTG TCTTATCATG CATATAAAGA AATTGAGGAC
      TTTCTCTTCA GTTTACACAC AGAATAGTAC GTATATTTCT TTAACCTCTG
-----
12051  AAACATCCTC AACATATTAA ACTGCAGGAT TGGATCCCCA CACCAGAGGA
      TTTGTAGGAG TTGTATAATT TGACGTCCTA ACCTAGGGGT GTGGTCTCCT
-----
12101  AATGAGTAAG TCACTCTGTA AAAGACTTAT TTTATGTGGA TTGTATAGTG
      TTACTCATTC AGTGAGACAT TTTCTGAATA AAATACACCT AACATATCAC
-----
12151  CAGAAAAGGC CTCAGAGATT TTAAGGATGC CTTTTACAGT TTCTGGGGAA
      GTCTTTTCCG GAGTCTCTAA AATTCCTACG GAAAATGTCA AAGAACCCTT
-----
12201  CAATCAGATA CTGACCCTGA CTGTTTTTATT GTAAGCTATA CATGTATATT
      GTTAGTCTAT GACTGGGACT GACAAAATAA CATTGATAT GTACATATAA
-----
12251  TTGTGATGCT GTAATACATG ATCCCATGCC CATAAGATGG GATCCTGAAG
      AACACTACGA CATTATGTAC TAGGGTACCG GTATTCTACC CTAGGACTTC
-----
12301  TTGGAATTTG GGTAAAATAT AAACCCCTCA GAGGAATTGT TGGATCTGCT
      AACCTTAAAC CCATTTTATA TTTGGGGAGT CTCCTTAACA ACCTAGACGA
-----

```

```

12351  GTGTTTATTA  TGCATAAACA  TCAAAGAAAC  TGTTCTCTTG  TTAAACCTTC
      CACAAATAAT  ACGTATTTGT  AGTTTCTTTG  ACAAGAGAAC  AATTGGAAG
-----
12401  TACCAGTCGC  TCAGAAGGTC  CAAAACCAAG  ACCTAGGCAC  GATCCTGTCC
      ATGGTCAGCG  AGTCTTCCAG  GTTTTGGTTC  TGGATCCGTG  CTAGGACAGG
-----
12451  TTCGATGTGA  CATGTTTGAA  AAGCATCACA  AGCCTCGGCA  GAAACGACCC
      AAGCTACACT  GTACAAACTT  TTCGTAGTGT  TCGGAGCCGT  CTTTGTGGG
-----
12501  AGGAGACGAT  CCATCGATAA  TGAGTCATGT  GCTTCCAGTA  GTGACACCAT
      TCCTCTGCTA  GGTAGCTATT  ACTCAGTACA  CGAAGGTCAT  CACTGTGGTA
-----
12551  GGCCAATGAG  CCAGGATCAC  TATGCACCAA  CCCTCTTTGG  AATCCTGGAC
      CCGGTTACTC  GGTCCTAGTG  ATACGTGGTT  GGGAGAAACC  TTAGGACCTG
-----
12601  CGCTACTATC  AGGGCTACTT  GAAGAGTCCA  GCAACCTACC  AAACTTGGA
      GCGATGATAG  TCCCGATGAA  CTTCTCAGGT  CGTTGGATGG  TTTGAACCTT
-----
12651  GTTCACATGT  CAGGTGGACC  CTTCTGGGAA  GAGGTTTATG  GGGACTCAAT
      CAAGTGTACA  GTCCACCTGG  GAAGACCCTT  CTCAAATAC  CCCTGAGTTA
-----
12701  TTTGGGTCCC  CCCTCTGGGT  CAGGTGAACA  TTCAGTTTTA  TAAGAATTAT
      AAACCCAGGG  GGGAGACCCA  GTCCACTTGT  AAGTCAAAAT  ATTCTTAATA
-----
12751  CAGATTCTAA  CTTGCTGTCA  GGCTGTAGAT  CTTCTTAAGC  TTGCGGGAGA
      GTCTAAGATT  GAACGACAGT  CCGACATCTA  GAAGAATTCT  AACGCCCTCT
-----

```

ApaI

```

12801  CGTCGAGTCC  AACCCCTGGG  CCGATATCCC  CATGGTGAGC  AAGGGCGAGG
      GCAGCTCAGG  TTGGGACCCG  GGCTATAGGG  GTACCACTCG  TTCCCGCTCC
-----
12851  AGCIGTTTAC  CGGGGTGGTG  CCCATCCTGG  TCGAGCTGGA  CGGCGACGTA
      TCGACAAGTG  GCCCCACCAC  GGGTAGGACC  AGCTCGACCT  GCCGCTGCAT
-----
12901  AACGGCCACA  AGTTCAGCGT  GTCGGGCGAG  GGCGAGGGCG  ATGCCACCTA
      TTGCCGGTGT  TCAAGTCGCA  CAGGCCGCTC  CCGCTCCCGC  TACGGTGGAT
-----
12951  CGGCAAGCTG  ACCCTGAAGT  TCATCTGCAC  CACCGGCAAG  CTGCCCCGTG
      GCCGTTTCGAC  TGGGACTTCA  AGTAGACGTG  GTGGCCGTTC  GACGGGCACG
-----
13001  CCTGGCCCCA  CCTCSTGACC  ACCTTCACCT  ACGGCGTGCA  GTGCTTEAGC
      GGACCGGGTG  GGAGCACTGG  TGAAGTGGA  TGCCGCACGT  CACGAAGTCG
-----
13051  CGCTACCCCG  ACCACATGAA  GCAGCACGAC  TTCTTCAAGT  CCGCCATGCC
      GCGATGGGGC  TGGTGTACTT  CGTCGTGCTG  AAGAAGTTCA  GCGGTTACGG
-----
13101  CGAAGGCTAC  GTCCAGGAGC  GCACCATCTT  CTTCAAGGAC  GACGGCAACT
      GCTTCCGATG  CAGGTCCTCG  CGTGGTAGAA  GAAGTTCCTG  CTGCCGTTGA
-----
13151  ACAAGACCCG  CGCCGAGGTG  AAGTTCGAGG  GCGACACCCT  GGTGAACCGC
      TGTTCGGGC  GCGGCTCCAC  TTCAAGCTCC  CGCTGTGGGA  CCACTTGGCG
-----
13201  ATCGAGCTGA  AGGGCATCGA  CTTCAAGGAG  GACGGCAACA  TCCTGGGGCA
      TAGCTCGACT  TCCCGTAGCT  GAAGTTCCTC  CTGCCGTTGT  AGGACCCCGT
-----
13251  CAAGCTGGAG  TACAACTACA  ACAGCCACAA  CGTCTATATC  ATGGCCGACA
      GTTCGACCTC  ATGTTGATGT  TGTCGGTGTT  GCAGATATAG  TACCGGCTGT
-----

```

```

13301 AGCAGAAGAA CGGCATCAAG GTGAACTTCA AGATCCGCCA CAACATCGAG
      TCGTCTTCTT GCCGTAGTTC CACTTGAAAGT TCTAGGCGGT GTTGTAGCTC
-----
13351 GACGGCAGCG TGCAGCTCGC CGACCACTAC CAGCAGAACA CCCCCATCGG
      CTGCCGTCGC ACGTCGAGCG GCTGGTGATG GTCGTCTTGT GGGGGTAGCC
-----
13401 CGACGGCCCC GTGCTGCTGC CCGACAACCA CTACCTGAGC ACCCAGTCCG
      GCTGCCGGGG CACGACGACG GGCTGTTGGT GATGGACTCG TGGGTCAGGC
-----
13451 CCCTGAGCAA AGACCCCAAC GAGAAGCGCG ATCACATGGT CCTGCTGGAG
      GGGACTCGTT TCTGGGGTTG CTCTTCGCGC TAGTGTACCA GGACGACCTC
-----
13501 TTCGTGACCG CCGCCGGGAT CACTCTCGGC ATGGACGAGC TGTACAAGTA
      AAGCACTGGC GGCGGCCCTA GTGAGAGCCG TACCTGCTCG ACATGTTTAT
-----

```

NotI

```

-----
13551 AAGCGGCCGC GACTCTAGGG GATTGCGCAT AAGTAAGTAA GCTTATGGAC
      TTCGCCGGCG CTGAGATCCC CTAAGCGCTA TTCATTCAAT CGAATACCTG
-----
13601 CTCAGAGAGG AAGTAACGAG GAGAGGGTGT GGTGGAATGT CACTAGAAAC
      GAGTCTCTCC TTCATTGCTC CTCTCCACCA CCACCTTACA GTGATCTTTG
-----
13651 CAGGGAAAAC AAGGAGGAGA GTATTACAGG GAAGGAGGTG AAGAACCTCA
      GTCCCTTTTG TTCCTCCTCT CATAATGTCC CTTCTCCAC TTCTTGAGT
-----
13701 TTACCCAAAT ACTCCTGCTC CTCATAGACG TACCTGGGAT GAGAGACACA
      AATGGGTTTA TGAGGACGAG GAGTATCTGC ATGGACCCTA CTCTCTGTGT
-----
13751 AGGTTCTTAA ATTGTCCTCA TTCGCTACTC CCTCTGACAT CCAACGCTGG
      TCCAAGAATT TAACAGGAGT AAGCGATGAG GGAGACTGTA GGTGCGACC
-----
13801 GCTACTAAAG CATTGCCTTA TGGCTGGAAA GTGGTCACCG AAAGCGGAAA
      CGATGATTTT GTAACGGAAT ACCGACCTTT CACCAGTGGC TTTCGCCTTT
-----
13851 TGATTATACT AGCCGCAGAA AGATCAGAAC ATTGACAGAG ATGACTCAGG
      ACTAATATGA TCGGCGTCTT TCTAGTCTTG TAACTGTCTC TACTGAGTCC
-----
13901 ATGAAATTAG AAAAAGGTGG GAAAGTGGAT ATTGTGACCC CTTTATTGAC
      TACTTTAATC TTTTCCACC CTTTCACCTA TAACACTGGG GAAGTAACTG
-----
13951 TCAGGAAGTG ACTCAGATGG ACCCTTCTAA AAGCCACAGA CAGTAAAAAT
      AGTCCTTCAC TGAGTCTACC TGGGAAGATT TTCGGTGTCT GTCATTTTAA
-----
14001 GTGTTAGCAC TTTATACAAT ATTATATCTG CTTAAGCTAT AGAAGCTTTC
      CACAATCGTG AAATATGTTA TAATATAGAC GAATTGATA TCTTCGAAAG
-----
14051 ACATACTCAG TAGCTGTTTC ACAATCAACA AAACAATGAT GATGTAATCA
      TGTATGAGTC ATCGACAAAG TGTTAGTTGT TTTGTTACTA CTACATTAGT
-----
14101 TAAGGAAGTA GTTTAAATAG GTTAATAAGT TTATTAGTTA TATAGAAAAT
      ATTCTTTCAT CAAATTTATC CAATTATTCA AATAATCAAT ATATCTTTA
-----
14151 AATATAGGAT AAAGTATAAG GATTAAGGTA TGAGGTGTGT GGCTCAACAC
      TTATATCCTA TTTCATATTC CTAATTCCAT ACTCCACACA CCGAGTTGTG
-----
14201 GTAGGGTGAC AAGAAAATCT ACTGTAATAG GACACAACAC CTCTAAAGTT
      CATCCCACTG TTCTTTTAGA TGACATTATC CTGTGTTGTG GAGATTTCAA
-----

```

SEQ ID NO: 2

```

a tgggtgcgag agcgtcggtta ttaagcgggg gagaattaga
  taaatgggaa
841 aaaattcggt taaggccagg gggaaagaaa caatataaac taaaacatat
agtatgggca
901 agcagggagc tagaacgatt cgcagttaat cctggccttt tagagacatc
agaaggctgt
961 agacaaatac tgggacagct acaaccatcc cttcagacag gatcagaaga
acttagatca
1021 ttatataata caatagcagt cctctattgt gtgcatcaaa ggatagatgt
aaaagacacc
1081 aaggaagcct tagataagat agaggaagag caaaacaaaa gtaagaaaaa
ggcacagcaa
1141 gcagcagctg acacaggaaa caacagccag gtcagccaaa attaccctat
agtgcagaac
1201 ctccaggggc aaatggtaca tcaggccata tcacctagaa ctttaaattgc
atgggtaaaa
1261 gtagtagaag agaaggcttt cagcccagaa gtaataccca tgttttcagc
attatcagaa
1321 ggagccaccc cacaagattt aaataccatg ctaaacacag tggggggaca
tcaagcagcc
1381 atgcaaatgt taaaagagac catcaatgag gaagctgcag aatgggatag
attgcacca
1441 gtgcatgcag ggcctattgc accaggccag atgagagaac caaggggaag
tgacatagca
1501 ggaactacta gtacccttca ggaacaaata ggatggatga cacataatcc
acctatccca
1561 gtaggagaaa tctataaaag atggataatc ctgggattaa ataaaatagt
agaatgtat
1621 agccctacca gcattctgga cataagacaa ggaccaaaag aacccttttag
agactatgta
1681 gaccgattct ataaaaactct aagagccgag caagcttcac aagaggtaaa
aaattggatg
1741 acagaaacct tgttggtcca aaatgcgaac ccagattgta agactatttt
aaaagcattg
1801 ggaccaggag cgacactaga agaaatgatg acagcatgtc agggagtggg
gggacccggc
1861 cataaagcaa gagttttggc tgaagcaatg agccaagtaa caaatccagc
taccataatg
1921 atacagaaaag gcaatttttag gaaccaaaga aagactgtta agtgtttcaa
ttgtggcaaa
1981 gaagggcaca tagccaaaaa ttgcagggcc cctaggaaaa agggctgttg
gaaatgtgga
2041 aaggaaggac accaaatgaa agattgtact gagagacagg ctaatttttt
agggaagatc
2101 tggccttccc acaagggaag gccagggaat tttcttcaga gcagaccaga
gccaacagcc
2161 ccaccagaag agagcttcag gtttggggaa gagacaacaa ctccctctca
gaagcaggag
2221 ccgatagaca aggaactgta tccttttagct tccctcagat cactctttgg
cagcgacccc
2281 tcgtcacaat aa

```

SEQ ID NO: 3

MGARASVLSGGELDKWEKIRLRPGGKKQYKLKHIVWASRELERFAVNPGLLE
TSEGRQILGQLQPSLQTGSEELRSLYNTIAVLYCVHQRIDVKDTKEALDKIEEE

QNKSKKKAQQAAADTGNNNSQVSQNYPIVQNLQGQMVHQAI SPRTLNAWVKV
VEEKA FSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDR LHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQANANPDCKTILKALGPATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNPATIMI QKGNFRNQRKTVKCFNCGKEGHI AKNCRAPRKKGCWKC GKEGH
QMKDCTERQANFLGKIWPSHKGRPGNFLQSRPEPTAPPEESFRFGEETTPSQK
QEPIDKELYPLASLRSLFGSDPSSQ

SEQ ID NO: 4

MGARASVLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLE
TSEGCRQILGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEE
QNKSKKKAQQAAADTGHS SQVSQNYPIVQNIQGQMVHQAI SPRTLNAWVKV
VEEKA FSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNSATIMMQRGNFRNQRKIVKCFNCGKEGHIARNCRAPRKKGCWKC GKEG
HQMKDCTERQANFLGKIWPSYKGRPGNFLQSRPEPTAPPFLQSRPEPTAPPEES
FRSGVETTPSQKQEPIDKELYPLTSLRSLFGNDPSSQ

SEQ ID NO: 5

MGARASVLSGGELDRWEKVRLRPGGKKKYKLKHIVWASRELERFAVNPGLLE
TSEGCRQILGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEE
QNKSKKKAQQAAADTGHS SQVSQNYPIVQNIQGQMVHQAI SPRTLNAWVKV
VEEKA FSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNSATIMMQRGNFRNQRKIVKCFNCGKEGHIARNCRAPRKKGCWKC GKEG
HQMKDCTERQANFLGKIWPSYKGRPGNFLQSRPEPTAPPFLQSRPEPTAPPEES
FRSGVETTPSQKQEPIDKELYPLTSLRSLFGNDPSSQ

SEQ ID NO: 6

TCC GGG CCC GGA ATG CCT ATA GTC CAG AAC ATC C

SEQ ID NO: 7

GCG GCC GCG TTT TGA GAA CGA AAT ACC GG

SEQ ID NO: 8

SEQ ID NO:1 with SEQ ID NO: 2 inserted between 12816 and 13552.

CORRECTED VERSION

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 January 2004 (08.01.2004)

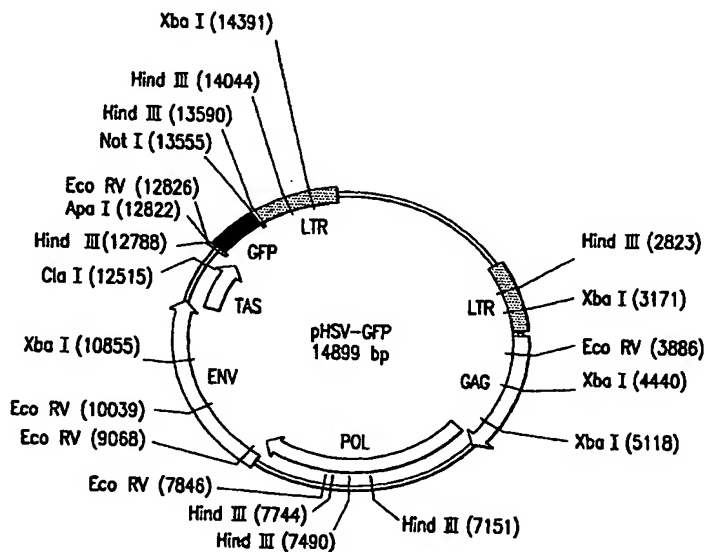
PCT

(10) International Publication Number
WO 2004/003153 A2

- (51) International Patent Classification⁷: C12N
- (21) International Application Number: PCT/US2003/020325
- (22) International Filing Date: 27 June 2003 (27.06.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/392,630 27 June 2002 (27.06.2002) US
- (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as REPRESENTED BY THE SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES CENTERS FOR DISEASE CONTROL AND PREVENTION [US/US]; Technology Transfer Office 4770 Buford Highway, (K-79), Atlanta, GA 30341 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): FOLKS, Thomas, M. [US/US]; 3815 Belle Glade Trail, Lithonia, GA 30039 (US). CHEN, Irvin, S., Y. [CN/US]; 1617 Margate Road, Palos Verde Estates, CA 90274 (US).
- (74) Agents: NEEDLE & ROSENBERG, P.C. et al.; Suite 1000, 999 Peachtree Street, Atlanta, GA 30309-3915 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,

[Continued on next page]

(54) Title: LIVE REPLICATING SPUMAVIRUS VECTOR



(57) Abstract: The present invention provides a vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid. The present invention also provides methods of treating or preventing a condition resulting from a viral, bacterial, or parasitic infection in a subject comprising administering to the subject an effective amount of the vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid. Also provided in the present invention are methods of treating a condition resulting from a cancer in a subject comprising administering to the subject an effective amount of the vector or vector containing composition comprising a spumavirus backbone and an antigen-encoding nucleic acid.



SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

(48) Date of publication of this corrected version:

21 May 2004

(15) Information about Corrections:

see PCT Gazette No. 21/2004 of 21 May 2004, Section II

Previous Correction:

see PCT Gazette No. 14/2004 of 1 April 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

LIVE REPLICATING SPUMAVIRUS VECTOR

BACKGROUND OF THE INVENTION

- 5 1. Spumavirus, also known as foamy virus for the characteristics of vacuolization the virus induces in cell culture, belongs to a distinct group of retroviruses. The simian foamy viruses (SFVs) include isolates from Old World and New World monkeys and are classified into 10 different serotypes based on serological cross-reactivities. Virus appears to persist in the host for a long period of time in a
10 latent form and can exist in the presence of neutralizing antibody.
2. Currently the most studied retrovirus, Human Immunodeficiency Virus, is believed to be derived from nonhuman primate transmission into humans at some past time. Concerns about the risk of transmission of retroviruses from non-human primates to humans working in research laboratories were heightened in the early 1990's when
15 two persons developed antibodies to SIV (Simian Immunodeficiency Virus) following work-related exposures, one of whom had clear evidence of persistent viral infection. (See CDC anonymous survey for simian immunodeficiency virus (SIV) seropositivity in SIV laboratory researchers – United States, 1992. MMWR Morb. Mort. Wkly. Rep. 1992; 41:814-5; Khabbaz R.F., et al. Brief report: infection of a laboratory worker with
20 simian immunodeficiency virus. New Eng. J. Med. 1994; 330:172-7; Khabbaz R.F., et al. Simian immunodeficiency virus needle stick accident in a laboratory worker. Lancet 1992; 340:271-3; and CDC. Guideline to prevent simian immunodeficiency virus infection in laboratory workers and animal handlers. MMWR 1988; 37:693-704.) In addition to SIV, nonhuman primate species used in biomedical research are
25 commonly infected with SFV (simian foamy virus), STLV (simian t-cell lymphotropic virus), and/or type D retroviruses. All of these retroviruses cause lifelong infections in nonhuman primates, and some are known to be transmissible through sexual contact, blood, or breast-feeding. Natural SFV infections in non-human primates have not been definitively associated with disease. In non-human primates, infection with the other
30 retroviruses may result in a clinical spectrum ranging from asymptomatic infection to life threatening immunodeficiency syndromes or lymphoproliferative disorders. The transmission routes of SFVs among non-human primates remain undefined, but the prevalence of seroreactivity is high among captive adult non-human primates.

3. Recent publications indicate that earlier serological tests showing human spumavirus antibodies in the human populating were incorrect. Immunological investigation of a previously reported human spumavirus revealed that it shared common antigens in complement fixation, immunofluorescence and neutralization assays with the chimpanzee foamy virus, SFV-6. Furthermore, failure to detect serological evidence of HFV infection in people from a wide geographical area suggested that this virus isolate was a variant of SFV-6 particularly since sera from chimpanzees naturally infected with SFV-6 neutralized both viruses. In a survey for prevalence of human foamy virus in more than 5000 human sera, collected from geographically diverse populations, none of the serum samples were confirmed as positive. Taken together with sequence analysis endorsing the phylogenetic closeness of the purported human spumavirus to SFV-6/7, these data strongly suggest that human foamy virus is not naturally found in the human populations. (See Ali, M. et al., "No Evidence of Antibody to Human Foamy Virus in Widespread Human Populations," AIDS Research and Human Retroviruses, Vol. 12, NO. 15, 1996).

4. Gene therapies have long looked for a good vector that can transport the foreign gene of choice into human cells. Thus, compositions and methods for gene therapy are needed that use a vector capable of carrying a significant amount of foreign DNA that will enter the host organism and not cause disease.

5. Compositions and methods for vaccination using recombinant live retroviruses are also needed. A live virus, that causes no illness in humans, and that has genes of antigens of choice incorporated into its genome, would provide for an excellent vaccination tool as a vector. The retrovirus would reproduce in the human host and expose the immune system to antigens so that an immune response can be initiated.

6. Targeted attack on reproducing cells is a goal of cancer treatment. What is needed are compositions and methods for cancer treatment that are specific for dividing cells that do not cause systemic damage to the cancer patient. A viral vector that could infect and kill dividing cells, without killing other cells of the host would provide a solution for cancer treatment.

7. The lack of any known disease associated with the virus from which the vector of the present invention was derived makes the present invention ideal for gene therapy regimens.

SUMMARY OF THE INVENTION

5 8. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to live replicating retroviral vectors and methods of their use.

9. Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be
10 learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

15 10. The present invention provides compositions comprising live replicating retroviral vectors, wherein the vector is derived from a spumavirus, and wherein the vector further comprises a nucleic acid that encodes a non-spumavirus peptide, polypeptide, or protein. Thus, the present invention provides compositions comprising live replicating spumavirus vectors.

20 11. Also provided by the present invention is a method of treating a subject with a condition, wherein the condition can be a viral infection, bacterial infection, parasitic infection, proliferative disorder (eg. cancer), or a condition associated with a genetic or autoimmune disorder; comprising administering to the subject a live replicating viral vector, wherein the immunizing construct is specific for the condition.

25 12. Also provided by the present invention is a method of preventing a condition in a subject, wherein the condition can be a viral infection, bacterial infection, parasitic infection, proliferative disorder, or a condition associated with a genetic or autoimmune disorder; comprising administering to the subject a live replicating viral vector, wherein the antigen-encoding nucleic acid is specific for the condition.

30 13. Also provided are methods of using the present vector for making models and using models to study diseases and potential treatments, as well as the models themselves.

BRIEF DESCRIPTION OF THE DRAWINGS

14. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

5 15. Figure 1 shows a map of the pHSV vector detailing the location of the spumavirus *env*, *pol*, *gag*, and *bel-1 (tas)* genes. The map also indicates the presence of LTR flanking the coding region for the spumavirus genes and the presence and location of *Apal* and *Not1* restriction sites.

10 16. Figure 2 shows a detailed restriction map of the pHSV vector. The sequence of the vector is provided in SEQ ID NO: 1.

17. Figure 3 shows a linear map of pHSV with the p17/24 sequence incorporated in the vector to generate a pHSV-HIV-gag p17/24 vector. This shows the location of p17/24 within the vector.

15 18. Figure 4 shows that several TA-p17/24 clones have the desired length fragment when cut with the *Apal* and *Not1* restriction enzymes. Shown is a 1% Acrylamide gel of purified clones resulting the transfection of p17/24 into the TA cloning vector (pCR2.1). Lane 1 is the marker BSTE II. Lane 2 is an empty pHSV vector used as a negative control. Lanes 3-7 are clones 1-5.

20 19. Figure 5 shows that p24 expression can be measured by ELISA in clones 2, 3, and 4 at days 2, 4, and 6 days post infection of BHK cells. Samples were also measured for p24 expression 6 days post-infection following freeze/thaw (F/T) of the clones.

20. Figure 6 shows that p24 expression is pronounced 5 days post infection in clone 2, clone 3 and clone 4.

25 21. Figure 7 shows that expression levels of p24 are maintained at high levels even on subsequent passages. Second pass clone 2 is diluted 10-fold and dilution was used to infect BHK cells. Supernatants were removed at 3, 7, and 10 days post infection and measured via ELISA for p24 expression.

30 22. Figure 8 shows Western blot analysis of clone 2. Lane 1 is empty pHSV vector, lane 2 is mock infected BHK cells, and lane 3 is BHK cells infected with clone 2. Samples were probed with LTF001, anti-p24, or anti-HIV serum and developed.

DETAILED DESCRIPTION

22. The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

23. Before the present compounds, compositions, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods or specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Definitions

24. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

25. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

26. In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

27. "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

Vectors

28. Disclosed are live replicating human spumavirus vectors suitable for human use comprising an immunizing construct, wherein the immunizing construct is inserted

in the *bet* gene. The disclosed immunizing construct can be an antigen-encoding nucleic acid.

29. Where reference is made to "antigen"-encoding nucleic acid, it is understood that in the context of the invention antigens encoded by the antigen-encoding nucleic acid can include but are not limited to immunogenic or non-immunogenic peptides, polypeptides, proteins, enzymes, cytokines. These antigens can be non-human exogenous antigenic sequences from viruses, bacteria, or parasites. The antigens can also be antigenic endogenous human or human derived sequences from a condition such as a cancer. Also, peptides encoded by the antigen-encoding nucleic acid can include non-antigenic sequences for the purposes of gene therapy.

30. Also disclosed are vectors of the invention, wherein the vector is pHSV or pHSV-GFP. Though at any one time reference may only be made to one of the vectors, it is an embodiment of the invention that both vectors may be used interchangeably and with equivalent results. Accordingly terms referring to either vector should be understood to refer to both vectors. Similarly, reference throughout this disclosure is given to the pHSV vector containing an HIV antigen-encoding nucleic acid. It is understood that this construct may be referred to as pFOV-gag, pFOV7-gag, pFOV-7-gag, pFOV7-p17/24, pFOV-7-p17/24, pFOV-p17/24, pFOV-7GFP-gag, pFOV-7-GFP-gag, pFOV-7GFP-p17/24, pFOV-7-GFP-p17/24, pFOV-7 HIV-gag p17/24, pHSV-gag, pHSV-p17/24, pHSV-GFP-gag, pHSV-GFP-p17/24 or pHSV-HIV-gag-p17/24. A vector of the invention, can have the sequence of pHSV-HIV-GAG p17/24. Thus, herein disclosed is a pHSV vector of the invention comprising SEQ ID NO: 1, wherein an antigenic construct comprising the *gag* gene of HIV-1 (SEQ ID NO: 2) has been inserted into the sequence at the unique *Apa* I site at 12816 and the unique *Not* I site at 13552. Said insertion resulting in the excision of nucleotides 12817-13551 of the vector and therefore partially replacing the *bet* gene of pHSV with SEQ ID NO: 2 (SEQ ID NO: 8). Therefore, also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is HIV-GAG p17/p24. Herein, p17/24 and p41 are used synonymously and are intended to refer to DNA encoding Gag or an antigenic fragment of Gag. It is also herein contemplated that other genes from HIV may be used as the antigen-encoding nucleic acid in the vector of the invention. Such genes can include the *env* and *pol* genes of HIV as well as *vpu*, *vif* and *nef* genes.

31. Also disclosed are vectors of the invention, wherein the vector has the sequence of pHSV, which is defined by the restriction map shown in Figures 1, 2, and 3. It is an embodiment of the present invention that modifications can be made to the pHSV vector to incorporate additional unique restriction sites throughout the vector including but not limited to within and around the bel-2(bet), pol, gag, env, or bel-1 genes. It is also understood that such modifications may provide additional features including but not limited to reporter function, resistance to a pharmaceutical agent, or enhancers. Related, but distinct vectors are disclosed in U.S. Patent No. 5,646,032 which is incorporated in its entirety herein by reference.

32. It is well-known in the art that vaccinations can be used prophylactically for the prevention of infections as well as therapeutically for the treatment of ongoing conditions. Such infections or conditions can be but are not limited to viral infections. Thus, also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an antigen from a virus. The viral antigen can be selected from the group of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2, and Simian Immunodeficiency virus (SIV). Also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is SIV-GAG. The art is replete with examples of viral antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

33. Vaccinations are also known for the prevention of bacterial infections. Additionally, antibiotics are well-known in the art for the treatment of various bacterial infections. Herein contemplated and disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an antigen from a bacterium. The bacterial antigen
5 can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella*
10 *haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*,
15 *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species. The art is replete with
20 examples of bacterial antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

34. The vectors of the invention are not limited to bacteria and viruses. Also disclosed are vectors of the invention, wherein the antigen-encoding nucleic acid is an
25 antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*. The art is replete with examples of parasitic
30 antigens whose sequences and methods of obtaining them are well known. Thus, a pHSV vector expressing these antigens is within the scope of the present invention.

35. The treatment of various forms of cancer is a major concern for millions of people worldwide and the focus of much of medical research. Herein contemplated are methods of treating a cancer comprising administering to a subject the vector of the invention. Therefore, also disclosed are vectors of the invention, wherein the antigen-
 5 encoding nucleic acid is a tumor antigen. The tumor antigen can be selected from the list consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmell7, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE,
 10 GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides. Each of these antigens is known and has a coding sequence that is publically available and well-known in the art.
 15 Further cancer antigens, whether later discovered or presently known can be expressed by the present pHSV vector as described herein.

36. There are instances wherein it is advantageous to administer the vector of the invention in a pharmaceutical composition that comprises other vaccines. Pharmaceutical compositions comprising multiple vaccines can be for therapeutic or
 20 prophylactic purposes. An example Examples of such a composition is other vaccines include the mumps, measles, rubella (MMR) vaccine, and vaccines against *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella*
 25 *pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*,
 30 other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*,

Neisseria gonorrhea, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species .

Specifically contemplated and disclosed are pharmaceutical compositions comprising
5 the vector of the invention and one or more additional vaccines. Also disclosed and
herein contemplated are instances in which the vector of the invention comprises more
than one antigen-encoding nucleic acid. In such a situation the vector of the invention
will produce each antigen encoded in the vector as a separate antigen.

37. There are instances in which a vector of the invention alone may not be
10 suitable for a given purpose (eg., A kit designed to screen potential drugs for the
treatment of a condition such kit, intended for use in laboratories without the
capabilities to transfect a cell-line with the vector). In such cases, cells previously
transfected with the vector of the invention are needed. Thus, also disclosed are cells
comprising the vector of the invention.

15 38. In an embodiment of the invention the antigen-encoding nucleic acid can
encode a non-antigenic sequence of DNA. This sequence can provide a functional
copy of a disrupted, mutated, disregulated or deleted gene. Examples of nucleic acids
encoding proteins that play a role in genetic disorders are known in the literature
relating to genetic disorders which is incorporated herein by reference. Methods of
20 making these cells are described and exemplified herein and in the art.

39. The ability to detect the presence of a construct can be a desirable feature
of any vector. As such, vectors are often contain a marker to show that the construct of
interest has been delivered to the subject (eg. a cell), and once delivered, is being
expressed. A marker can take the form of a gene that is detectable when expressed.
25 Thus, also disclosed are vectors of the invention further comprising a reporter gene.
One example of a reporter gene is green fluorescence protein (GFP).

Compositions

40. The invention includes a composition comprising a vector of the invention.
Disclosed are the components to be used to prepare the disclosed compositions as well
30 as the compositions themselves to be used within the methods disclosed herein. These
and other materials are disclosed herein, and it is understood that when combinations,
subsets, interactions, groups, etc. of these materials are disclosed that while specific

reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular spumavirus vector or antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) is disclosed and discussed and a number of modifications that can be made to a number of molecules including the spumavirus vector or antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) are discussed, specifically contemplated is each and every combination and permutation of spumavirus vector and antigen-encoding nucleic acid (e.g., p17, p24, p17/24, gag, gp120) and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods. Often therapeutic agents and vaccines are administered in formulations or combinations that incorporate other therapeutic modalities or necessary components for the purposes of time release, delivery, or augmentation of a response. It is understood and herein contemplated that the disclosed vectors and exogenous nucleic acid of the invention can be combined and administered with any such modality or component.

Expression systems

41. The nucleic acid vectors of the invention that are delivered to cells typically contain expression controlling systems for controlling the expression of heterologous/exogenous nucleic acid. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the

transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

Viral Promoters and Enhancers

5 42. Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus, cytomegalovirus and most preferably spumavirus, or from heterologous mammalian promoters, e.g. beta actin promoter. Each of these promoters is known and has a
10 sequence that is publicly available. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a *HindIII* E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course,
15 promoters from the host cell or related species also are useful herein. Methods of functionally linking promoters with coding sequences are well-known.

 43. Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108
20 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements
25 that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred
30 examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

44. The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or
5 alkylating chemotherapy drugs.

45. In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular
10 type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

46. It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types
15 such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

47. Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are
20 transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification
25 and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the
30 above sequences improve expression from, or stability of, the construct.

Markers

48. The viral vectors can include nucleic acid sequence encoding a marker product (reporter gene). This marker product is used to determine if the gene has been delivered to the cell and once delivered, is being expressed. Marker genes can be but
5 are not limited to the *E. Coli* lacZ gene, which encodes β -galactosidase, and green fluorescent protein.

49. In some embodiments the marker may be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin.
10 When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells
15 and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking
20 the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

50. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These
25 schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under
30 eukaryotic control to convey resistance to the appropriate drug G418 or neomycin

(geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

Treatment and Prevention Methods

51. By "treating" is meant an improvement in the disease state (i.e., viral
5 infection, bacterial infection, parasitic infection, cancer, genetic disorder, or
autoimmune disease) is observed and/or detected upon administration of a substance of
the present invention to a subject. Treatment can range from a positive change in a
symptom or symptoms of the disease to complete amelioration of the viral infection,
bacterial infection, parasitic infection, or cancer (e.g., reduction in severity or intensity
10 of disease, alteration of clinical parameters indicative of the subject's condition, relief
of discomfort or increased or enhanced function), as detected by art-known techniques.
The methods of the present invention can be utilized to treat an established viral
infection, bacterial infection, parasitic infection, or cancer. One of skill in the art would
recognize that viral infection, bacterial infection, parasitic infection, or cancer refer to
15 conditions characterized by the presence of a foreign pathogen or abnormal cell growth.
Clinical symptoms will depend on the particular condition and are easily recognizable
by those skilled in the art of treating the specific condition. Treatment methods can
include, but are not limited to therapeutic vaccinations. Thus, Disclosed are methods of
treating a subject with a condition comprising administering to the vector of the
20 invention.

52. Also disclosed are methods of the invention, wherein the condition being
treated is a viral infection. The viral infection can be selected from the list of viruses
consisting of Herpes simplex virus type-1, Herpes simplex virus type-2,
Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6,
25 Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus,
Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus,
Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus,
Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma
30 virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern
Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A,

Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

53. Also disclosed are methods of the invention, wherein the antigen-encoding
5 nucleic acid is an antigen from a virus. The viral antigen can be selected from the group of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
10 virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
15 Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

54. Also disclosed are methods of the invention, wherein the condition being
20 treated is a bacterial infection. The bacterial infection can be selected from the list of bacterium consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other
25 *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter*
30 species, *Neisseria meningitidis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*, other

Pseudomonas species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

55. Also disclosed are methods of the invention, wherein the antigen-encoding
5 nucleic acid is an antigen from a bacterium. The bacterial antigen can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other
10 *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus*
15 *agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*,
20 and other *Yersinia* species.

56. Also disclosed are methods of the invention, wherein the condition being treated is a parasitic infection. The parasitic infection can be selected from the list of parasites consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma*
25 *cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

57. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium*
30 *vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

58. Also disclosed are methods of the invention, wherein the condition being treated is cancer.

59. The disclosed vectors and vector containing compositions can be used to treat any disease where uncontrolled cellular proliferation occurs such as cancers. A
5 non-limiting list of different types of cancers is as follows: lymphomas (Hodgkins and non-Hodgkins), leukemias, carcinomas, carcinomas of solid tissues, squamous cell carcinomas, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, or
10 cancers in general.

60. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphoma, B cell lymphoma, T cell lymphoma, mycosis fungoides, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma
15 of head and neck, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer, neuroblastoma/glioblastoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary
20 cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, or pancreatic cancer.

61. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is a tumor antigen. The tumor antigen can be selected from the list
25 consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pm117, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7,
30 prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

62. Disclosed are methods of treating a condition in a subject comprising administering to the subject the vector of the invention, wherein the condition is due to a mutated, dysregulated, disrupted, or deleted gene; autoimmunity; or inflammatory diseases.

5 63. Disclosed are methods of treating a condition in a subject, wherein the condition can be selected from list consisting of cystic fibrosis, asthma, multiple sclerosis, muscular dystrophy, diabetes, tay-sachs, spinobifida, cerebral palsy, parkinson's disease, lou gehrigg disease, alzheimer's, systemic lupus erythematosis, hemophilia, Addison's disease, Cushing's disease.

10 64. By "preventing" is meant that after administration of a substance of the present invention to a subject, the subject does not develop the symptoms of the viral, bacterial, or parasitic infection, and/or does not develop the viral, bacterial, or parasitic infection. "Preventing" or "prevention" can also refer to the ultimate reduction of an infection, condition, or symptoms of an infection, or condition relative to infections or
15 conditions in subjects that do not receive the substance. Methods of prevention can include, but are not limited to prophylactic vaccination. As such, disclosed are methods of preventing an infection in a subject comprising administering to the subject the vector of the invention.

 65. Also disclosed are methods of the invention, wherein the infection
20 prevented is a viral infection. The viral infection can be selected from the list of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E
25 virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus,
30 Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-

cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

66. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a virus. The viral antigen can be selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

67. Also disclosed are methods of the invention, wherein the infection prevented is a bacterial infection. The bacterial infection can be selected from the list of bacterium consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other

Hemophilus species, Clostridium tetani, other Clostridium species, Yersinia enterocolitica, and other Yersinia species.

68. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a bacterium. The bacterial antigen can be selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*,
 10 *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus*
 15 *agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

20 69. Also disclosed are methods of the invention, wherein the infection prevented is a parasitic infection. The parasitic infection can be selected from the list of parasites consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma*
 25 *mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

70. Also disclosed are methods of the invention, wherein the antigen-encoding nucleic acid is an antigen from a parasite. The parasitic antigen can be selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*,
 30 *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

71. Also disclosed are methods of the invention, wherein the subject is a horse, cow, pig, dog, cat, mouse, monkey, human, or a cell isolated from such an animal.

Delivery of the compositions to cells

72. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, and the present viral vectors. Appropriate means for transfection, including the present viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

73. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

74. As used herein, viral vectors such as pHSV are agents that transport the disclosed antigen-encoding nucleic acids, such as p17/24 into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. Retroviral vectors especially spumavirus vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors. A preferred embodiment is a viral vector which has been engineered so as to induce the immune response of the host organism, elicited by the peptides encoded on the vector.

75. Viral vectors can have higher transfection abilities (ability to introduce genes) than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural and structural genes, a polymerase, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA.

Constructs of this type can carry large fragments of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

76. Spumaviruses are retroviruses. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference. Although the present spumavirus vector is unique, the methods described for using other types of viral vectors can be useful in certain contexts. See for example U.S. Patent No. 5,646,032, which is incorporated herein for its teaching of those methods.

77. A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serves as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for large fragments of foreign sequence to be inserted into the viral genome, become

reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

5 78. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in *cis* by the
10 helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

In vivo/ex vivo

79. As described herein, the vector-containing compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the
15 subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

80. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The
20 compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known
25 for transplantation or infusion of various cells into a subject.

Nucleic Acid Delivery

81. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or RNA, or
30 the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the antibody-encoding DNA fragment is under the transcriptional regulation of a promoter, as would be well understood by one of ordinary skill in the art.

82. As one example, if the antigen-encoding nucleic acid of the invention is delivered to the cells of a subject in a spumavirus vector, the dosage for administration of spumavirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{10} to 10^{12} pfu per injection. In some cases lower dosages (eg., 10^3 , 10^4 , 10^5 , and 10^6 pfu) can be effective. A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at two, four, six month intervals (any intervening time intervals or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

83. Parenteral administration of the nucleic acid or vector of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

Delivery of pharmaceutical products

84. The vector or vector-containing compositions can be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

85. The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, subcutaneously, transdermally, extracorporeally, topically, gene gun or the like, although topical

intranasal administration or administration by inhalant is typically preferred. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. The latter may be effective when a large number of animals is to be treated simultaneously. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

86. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which

the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

10 **Pharmaceutically Acceptable Carriers**

87. The compositions, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

88. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

89. Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like.

90. The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed compositions can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, by gene gun, or transdermally.

91. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable

organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient
5 replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

92. Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional
10 pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

93. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

94. Some of the compositions may potentially be administered as a
15 pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic
20 acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanalamines.

Therapeutic Uses

95. The substances of the present invention can be delivered at effective
25 amounts or concentrations. An effective concentration or amount of a substance is one that results in treatment or prevention of the condition (e.g. HIV or AIDS). One skilled in the art would know how to determine an effective concentration or amount according to methods known in the art, as well as provided herein. One of skill in the art can
30 utilize *in vitro* assays to optimize the *in vivo* dosage of a particular substance, including concentration and time course of administration.

96. Herein, immune modulatory substances are described. As defined immune modulation refers to any change in the immune response. This includes but is not limited to increase or decreases in the number of antigen specific plasma cells, memory B cells, memory T cells, activated CD8 T cells, cytokine production, and cytotoxic killing or the maintenance of homeostatic levels of the same when the responses would otherwise be changing. The dosage ranges for the administration of the substances are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

97. For example, to evaluate the efficacy of treatment of humans with a condition, such as for example, HIV, with a substance that modulates normal immune responses to HIV, the following studies can be performed. Patients with active infection can be selected. Drug efficacy can be monitored via viral titer, antibody ELISA or ELISPOT and CD4 T cell count. Patients can be randomized to two different protocols. In one protocol, subjects can remain on initial medication and in the second protocol, subjects can have their medication tapered after receiving the substance that modulates immune responses.

98. In one embodiment, treatment can consist of either a single dosage of 0.3 mg to 0.6 mg/animal of the vector expressing a substance that treats or prevents the condition. Additionally, dosage as low as 30 μ g/animal to 300 μ g/animal can be used with intramuscular injection of infectious DNA. In one example, a DNA immunization comprising 0.3 mg of the pFOV-gag vector is administered to mouse via gene gun. After two weeks the subject is monitored using conventional assays to assess a generated immune response to p17/24 in the form of antibody production and antigen specific T cells. Having generated a response, the mouse is then challenged with an infectious dose of HIV. The mouse is then monitored for disease progression and clinical symptoms associated with HIV. Alternatively, a mouse can be infected with HIV and then given a therapeutic dose of the pFOV-gag. The animal is monitored for

changes in the viral lode, clinical progression of symptoms, as well as immune responses in comparison to non treated control animals. In a further example, a DNA immunization comprising 0.05mg (50µg) of the pFOV-gag vector is administered to mouse via intrauscular injection of infectious DNA. After two weeks the subject is

5 monitored using conventional assays to assess a generated immune response to p17/24 in the form of antibody production and antigen specific T cells. Having generated a response, the mouse is then challenged with an infectious dose of HIV. The mouse is then monitored for disease progression and clinical symptoms associated with HIV. Alternatively, a mouse can be infected with HIV and then given a therapeutic dose of

10 the pFOV-gag. The animal is monitored for changes in the viral lode, clinical progression of symptoms, as well as immune responses in comparison to non treated control animals. The art of determining dosage for an animal based on size is well known. It is understood that a skilled artisan would be able to determine the proper dosage of a substance for an animal based on the dosage of the same substance

15 administered to another animal of similar or different size. For example, whereas a 200g mouse would receive a 50µg dose of the immunization, a 150lb (68kg) human would be administered an immunization comprising about 17mg of the vector.

99. Disclosed are methods of detecting the expression of the vector of the invention comprising using a first antibody to the antigen to measure protein expression

20 in a quantitative or qualitative way, further comprising detecting the first antibody directly via a colorimetric measurement produced through the use of a substrate and a conjugated antibody or indirectly via a first antibody to the antigen which in turn is bound by a second antibody which is conjugated and will result in a colorimetric measurement when combined with a substrate.

25 100. Also disclosed are methods of the invention, wherein the antigen is detected by placing an aliquot of the vector of the invention in a lane on a gel and probing the gel for the antigen.

101. Disclosed are methods of detecting the expression of the vector of the invention comprising using a fluorescently labeled first antibody specific for the

30 antigen and visualizing the antigen using a flow cytometer or fluorescence microscope.

102. Also disclosed are methods of the invention, wherein the first antibody is not fluorescently labeled, but a target for a second antibody with a fluorescent label.

103. Disclosed are methods of detecting the expression of the vector of claim the invention comprising using cytolitic killing assay to assess activity.

104. Disclosed are methods of detecting the vector of the invention further comprising obtaining a sample from a subject comprising a tissue biopsy or removal of
5 blood or bone marrow.

Screening Methods

105. Also provided by the present invention is a method of screening a substance for effectiveness in treating or reducing the severity of the condition (e.g., HIV infection) comprising: a) obtaining an animal having the condition or
10 characteristic (e.g., symptom) of the condition; b) administering the substance to an animal having one or more characteristics of the condition; and assaying the animal for an effect on the condition, thereby identifying a substance effective in reducing the condition.

106. The ability of a substance to reduce the severity of a condition can be
15 determined by evaluating the histological and clinical manifestations, of the animal with condition before and after administration of the substance of interest and quantitating the amount of reduction of the condition.

107. The animal in which the condition or characteristic (e.g., symptom) of the condition is produced can be any mammal and can include but is not limited to
20 mouse, rat, guinea pig, hamster, rabbit, cat, dog, goat, monkey, and chimpanzee. The condition or characteristic (e.g., symptom) of the condition can be produced in the animal by any method known in the art. For example, HIV can be produced by introducing into the animal (eg., chimpanzee infected with HIV or rhesus macaques or nemestrina macaques infected with an HIV-1 env on an SIV backbone. Pullium , JK, et.
25 al., J. Infectious Dis. 183:1023, 2001) an infectious amount of HIV.

108. The present invention also provides a method of screening for a substance effective in preventing the condition (e.g., HIV infection) comprising: a) administering the substance to an animal susceptible to the condition; b) subjecting the animal to treatment that will induce the condition or characteristic (e.g., symptom) of
30 the condition; and c) assaying cells from the animal for an change in immune responses as compared to an the immune responses in a control animal having the condition in the

absence of the substance identifies a substance that is effective in preventing the condition.

109. For example, the methods of measuring the amount of p17/24 or other HIV antigen in an animal include, but are not limited to, ELISA, PCR, FACS analysis, reverse-transcriptase-polymerase chain reaction and ELISPOT, Northern blots, Southern blots, and Western blots.

110. A model for use in screening for substances effective in treating or preventing a disease comprising an animal capable of manifesting a characteristic of the disease is provided, wherein the animal has been administered the vector of the invention.

111. A method of making the model of the invention comprising obtaining administering to an animal capable of manifesting a characteristic of the disease and administering to said animal the vector of claim 1a vector of the invention which encodes an antigen associate with the disease.

112. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) administering the substance to the model of the invention; and b) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

113. A method of screening for a substance effective in preventing a disease associated with an immunizing construct comprising: a) administering a the vector of the invention to a subject; b) subjecting the subject to treatment that will induce the disease or characteristic (e.g., symptom) of the disease; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in preventing the disease.

114. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) subjecting the subject to treatment that will induce the disease or characteristic (e.g., symptom) of the disease; b) administering a the vector of the invention to a subject; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control

subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

Sequence similarities

115. It is understood that as discussed herein the use of the terms homology
5 and identity mean the same thing as similarity. Thus, for example, if the use of the
word homology is used between two non-natural sequences it is understood that this is
not necessarily indicating an evolutionary relationship between these two sequences,
but rather is looking at the similarity or relatedness between their nucleic acid
sequences. Many of the methods for determining homology between two
10 evolutionarily related molecules are routinely applied to any two or more nucleic acids
or proteins for the purpose of measuring sequence similarity regardless of whether they
are evolutionarily related or not.

116. In general, it is understood that one way to define any known variants
and derivatives or those that might arise, of the disclosed genes and proteins herein, is
15 through defining the variants and derivatives in terms of homology to specific known
sequences. This identity of particular sequences disclosed herein is also discussed
elsewhere herein. In general, variants of genes and proteins herein disclosed typically
have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87,
88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence
20 or the native sequence. Those of skill in the art readily understand how to determine
the homology of two proteins or nucleic acids, such as genes. For example, the
homology can be calculated after aligning the two sequences so that the homology is at
its highest level.

117. Another way of calculating homology can be performed by published
25 algorithms. Optimal alignment of sequences for comparison may be conducted by the
local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by
the homology alignment algorithm of Needleman and Wunsch, J. Mol Biol. 48: 443
(1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad.
Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms
30 (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package,
Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

118. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.*

183:281-306, 1989 which are herein incorporated by reference for at least material
5 related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

119. For example, as used herein, a sequence recited as having a particular
10 percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not
15 have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80
20 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using
25 each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

Nucleic acids

120. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example HIV-1 *gag*, as
30 well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for

example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of
5 nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

Sequences

121. There are a variety of sequences related to the *gag* gene that are publicly available (eg., Genbank Accession Number: L03707), these sequences and others are
10 herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

122. One particular sequence set forth in *gag* that is publicly available and having Genbank accession number L03707 is used herein, as an example, to exemplify the disclosed compositions and methods. It is understood that the description related to
15 this sequence is applicable to any sequence related to *gag* unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of *gag*). Primers and/or probes can be designed for any *gag* sequence given the information disclosed herein and known in
20 the art.

Peptides/ Protein variants

123. There are numerous variants of protein antigens that are antigenic. For example, there are variants of the *gag* protein and protein that are known and herein contemplated. In addition to the known functional HIV-1 *gag* strain variants there are
25 derivatives of the *gag* proteins which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal
30 fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein

derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion.

Deletions are characterized by the removal of one or more amino acid residues from the

5 protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at
10 predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in
15 adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been
20 removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1: Amino Acid Abbreviations

Amino Acid	Abbreviations
Alanine	Ala, A
Allosoleucine	Alle
Arginine	Arg, R
Asparagine	Asn, N
aspartic acid	Asp, D
Cysteine	Cys, C
glutamic acid	Glu, E
Glutamine	Gln, Q
Glycine	Gly, G
Histidine	His, H
Isoleucine	Ile, I
Leucine	Leu, L

Amino Acid	Abbreviations
Lysine	Lys, K
Phenylalanine	Phe, F
Proline	Pro, P
pyroglutamic acidp	Glu
Serine	Ser, S
Threonine	Thr, T
Tyrosine	Tyr, Y
Tryptophan	Trp, W
Valine	Val, V

TABLE 2: Amino Acid Substitutions	
Original Residue	Exemplary Conservative Substitutions, others are known in the art.
	Ala; Ser
	Arg; Lys, gln
	Asn; Gln; his
	Asp; Glu
	Cys; Ser
	Gln; Asn, lys
	Glu; Asp
	Gly; Pro
	His; Asn; gln
	Ile; Leu; val
	Leu; Ile; val
	Lys; Arg; gln;
	Met; Leu; ile
	Phe; Met; leu; tyr
	Ser; Thr
	Thr; Ser
	Trp; Tyr
	Tyr; Trp; phe
	Val; Ile; leu

124. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic

residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

125. The replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the antigenic polypeptides encoded and expressed by the vectors provided herein.

126. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

127. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

128. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:3 sets forth

a particular sequence of a *gag* protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. It is understood that those of skill in the art will recognize variants of a disclosed protein as being a variant. Particularly, there are numerous naturally occurring variants of viral (e.g., HIV) antigens. For example, it is understood that those of skill in the art would recognize that the particular *gag* protein disclosed in SEQ ID NO: 4 is a *gag* protein from a different HIV-1 isolate from the HIV-1 *gag* protein in SEQ ID NO: 3 and therefore a variant that can be used with equivalent results. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

129. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

130. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

131. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

132. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein

sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO: 3 is set forth in SEQ ID NO: 2. In addition, for example, a disclosed conservative derivative of SEQ ID NO: 4 is shown in SEQ ID NO: 5, where the isoleucine (I) at position 19 is changed to a valine (V). It is understood that for this mutation all of the nucleic acid sequences that encode this particular derivative of the *gag* gene of the LAI strain of HIV-1 are also disclosed. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular *gag* from which that protein arises is also known and herein disclosed and described.

Computer readable mediums

133. It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

Methods of making the compositions

134. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

5 Nucleic acid synthesis

135. For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for
10 example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Bioscience, Burlington, MA or ABI Model
15 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

20 136. Disclosed are cells produced by the process of transforming the cell with any of the disclosed nucleic acids. Disclosed are cells produced by the process of transforming the cell with any of the non-naturally occurring disclosed nucleic acids.

137. Disclosed are any of the disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the non-naturally
25 occurring disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the disclosed peptides produced by the process of expressing any of the disclosed non-natural nucleic acids.

138. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed
30 are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal

with any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

139. Also disclosed are animals produced by the process of adding to the animal any of the cells disclosed herein.

5 **Methods of using the compositions as research tools**

140. The disclosed vectors, compositions and methods can be used for targeted gene disruption and modification in any animal that can undergo these events. Gene modification and gene disruption refer to the methods, techniques, and compositions involved in the selective removal or alteration of a gene or stretch of
10 chromosome in an animal, such as a mammal, in a way that propagates the modification through the germ line of the mammal. In general, a cell is transformed with a vector which is designed to homologously recombine with a region of a particular chromosome contained within the cell, for example, as described herein. This homologous recombination event can produce a chromosome which has
15 exogenous DNA introduced, for example in frame, with the surrounding DNA. This type of protocol allows for very specific mutations, such as point mutations, to be introduced into the genome contained within the cell. Methods for performing this type of homologous recombination are disclosed herein.

141. One of the preferred characteristics of performing homologous
20 recombination in mammalian cells is that the cells should be able to be cultured, because the desired recombination events occur at a low frequency.

142. Once the cell is produced through the methods described herein, an animal can be produced from this cell through either stem cell technology or cloning technology. For example, if the cell into which the nucleic acid was transfected was a
25 stem cell for the organism, then this cell, after transfection and culturing, can be used to produce an organism which will contain the gene modification or disruption in germ line cells, which can in turn be used to produce another animal that possesses the gene modification or disruption in all of its cells. In other methods for production of an animal containing the gene modification or disruption in all of its cells, cloning
30 technologies can be used. These technologies generally take the nucleus of the transfected cell and either through fusion or replacement fuse the transfected nucleus with an oocyte which can then be manipulated to produce an animal. The advantage of

procedures that use cloning instead of ES technology is that cells other than ES cells can be transfected. For example, a fibroblast cell, which is very easy to culture can be used as the cell which is transfected and has a gene modification or disruption event take place, and then cells derived from this cell can be used to clone a whole animal.

5 143. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in
10 the sentence or section in which the reference is cited.

 144. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention
15 disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

Examples

 145. The following examples are put forth so as to provide those of ordinary
20 skill in the art with a complete disclosure and description of how the vectors, compounds, compositions, and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors
25 and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Example 1

 146. In order to obtain the p17/p24 fragment of the HIV-1 gag gene, pSX
30 plasmid containing the gag gene was subjected to 30 cycles of PCR using forward Apa1-p17 5' primer and reverse Not1-p24 3' primer. PCR product was cloned into a TA cloning vector (pCR2.1 Invitrogen; Carlsbad, CA) and expanded. Fig. 1 shows a

map of the empty pHSV vector. The vector contains the viral envelope gene *env* as well as its structural gene *gag* and viral polymerase gene *pol*. The vector also possesses a transactivator (TAS) gene *bel-1* and 2 LTR that flank the coding region of the vector. An inspection of the vector SEQ (SEQ ID NO: 1) shows where various restriction sites are located on the vector. A complete listing of the sites is found in Fig. 2 and these sites are shown in SEQ ID NO: 1.

147. Inspection of the restriction map revealed *Apal* and *NotI* unique restriction sites located around the BET (Bel-2) gene at 13522 and 12816 respectively. As such, the BET gene was chosen as the site for integration of the exogenous nucleic acid (Figure 3). The p17/p24 were excised from the TA cloning vector using *Apal* and *NotI* restriction enzymes. Similarly, the empty pHSV vector was cut with *Apal* and *NotI*.

148. The p17/p24 segment of *gag* (789 (start of p17) – 1876 (end of p24)) was ligated to pHSV and the pHSV-GAG was transformed into *E. coli* via electroporation. The bacteria was plated onto high Amp plates with the pHSV conferring *ampR* between the LTRs on the approximate 3kb plamid. Seven colonies were chosen and checked for insertion and proper orientation. Figure 4 shows that clone 2, 3, 4 when cut with *Apal* and *NotI* possessed a 1 kb fragment. This indicates that the p17/p24 fragment is intact and properly oriented.

149. Knowing that the construct was integrated into the vector, the next objective was to examine expression levels of the antigen. Integration is meaningless if the desired protein is not expressed. To rest expression of p17/p24, the loaded pHSV vector was transfected using lipofectamine into BHK cells and supernatants were collected at various time points post transfection. ELISA testing for p24 expression was conducted on the collected supernatants (Fig. 5).

150. Following transfection, clone 2 had clearly measurable p24 expression. The p24 expression level for clone 2 was increased at each time point after day 2, clones 3 and 4 showed no measurable expression of p24 until day 5 post transfection. Subsequent Elisa data revealed that 5 days post transfection clones 2, 3, and 4 could produce as much as 500 ng/ml, 1000ng/ml, and 800ng/ml of p24 respectively (Fig. 6). At the same time *in vitro* cytopathology was assessed. Cells from the day 2 cultures

had very little CPE. However, by day 5 CPE was readily observed. The presence of CPE shows that the p24 activity was associated with the replicating viral vector.

151. Fig. 7 shows p24 expression in clone 2 after 2nd pass. Supernatants were collected at 3, 7 and 10 days after infection with diluted virus. At day 3 no p24 is
5 observed. However, by day 7 1000ng/ml of p24 is observed at the 10⁻² dilution, which is confirmed at the 10⁻³ dilution with a measurement of 100ng/ml. By day 10, the 10⁻² to 10⁻⁴ dilutions all had approximately 200ng/ml of p24. As such, day 7 undiluted virus would yield 10⁵ ng/ml p24 and at day 10 the secretion level ranges from 2x10⁴ to 2x10⁶ ng/ml of p24. This is an extremely high expression level and indicates likely success *in*
10 *vivo* as this vector is resulting in expression comparable to the highest 25% of vectors.

152. As a verification that p24 Elisa data was actually measuring p17/p24 (p41) and not just p24, western blotting was performed (Fig. 8). Supernatants from BHK cells infected with clone 2 pHSV-gag, empty pHSV, or mock infected (infected with PBS) were run on a polyacrylamide gel and probed for p24, HIV, or LTF001 as a
15 positive control. LTF001 antibody bound to all 3 lanes. However, bands were apparent only in the lane for clone 2 when either anti-p24 or anti-HIV was used as a probe. The resulting bands show the smallest band to be 41kd, which corresponds to the size of p17/p24.

153. Histological observations were made from *in vitro* culture of pHSV in
20 BHK cells. Cells infected with pHSV-gag showed CPE; however, this CPE is not usually observed *in vivo*.

Methods

Cloning and PCR

154. The p17 and p24 segments of the LAI strain of HIV-1 gag were cloned
25 into a pHSV spumavirus vector. Briefly, pSX plasmid containing the LAI strain HIC-1 gag gene was obtained from the HIV repository. The pSX plasmid was used as a template for PCR amplification and cloning of p17 and p24. A forward Apa1-p17 5' primer TCC GGG CCC GGA ATG CCT ATA GTC CAG AAC ATC C (SEQ ID NO: 6) and a reverse Not1-p24 3' primer GCG GCC GCG TTT TGA GAA CGA AAT
30 ACC GG (SEQ ID NO: 7) were used to amplify p41. The amplified segment was cloned into a TA cloning vector (pCR2.1; Invitrogen Carlsbad, CA) and expanded.

Restriction digest of the TA-p41 vector with Apa1 and Not1 removed p17/24 from the vector. The p41 segment was then inserted into pHSV within the *bet* (*bel-2*) gene.

Transformation

155. The pHSV vector and p17/24 antigen were ligated together with ligase for 16hrs at 4C. Following ligation, pHSV-gag was electroporated into E. coli and incubated for 1hr at 37C in 2xyT broth media. The samples are then plated on high Ampicillin plates and incubated overnight at 37C. Colonies growing on the high Amp plates were selected and transferred to 2xyT broth media overnight at 37C. Minipreps were performed on the overnight cultures. Plasmids were cut with Apa1 and Not1 and aliquots were run on 1% agarose gel.

ELISA and Western Blot

156. ELISA and Western blot were performed using techniques commonly used in the art.

Example 2

157. Animal subjects can be used to screen the effectiveness of a pHSV vector and an antigen-encoding nucleic acid. Additionally, animal subjects may be used to study the vector-antigen combination's ability to prevent or treat a condition. The vector can also be used to induce a condition in an animal that is associate with a disease, and that animal can then be used to study the disease/condition or to study potential treatments for the disease/condition. Such conditions can be infections resulting from viruses, bacteria, or parasites; autoimmune reactions including inflammatory diseases, asthma, systemic lupus erythamatosi, muscular dystrophy, or multiple sclerosis, diabetes, tay-sachs, spinobifida, cerebral palsy, parkinson's disease, lou gehrigg disease, alzheimer's, hemophelia, Addision's disease, Cushing's disease; or cancer. Animals can include but are not limited to mice, rats, pig, dog, monkey, chimpanzee, and human.

158. Mice are injected with a sub-immunizing dose of pHSV-gag. 0.3mg/animal of pHSV-gag are administered via gene gun. This same immunizing dose may be administered by an alternative route such as intramuscular, intravenous, intraperitoneal, intracranial, or subcutaneous. Mice are then monitored and starting at 15 days post immunization mice are bled every three days to day 30 post immunization. Blood samples are collected for serum extraction and for the isolation of peripheral

blood lymphocytes (PBL). Serum samples can be used immediately for ELISA assays to look for antibody to the antigen encoded in the vector or for cytokine increases following immunization. Samples can also be frozen and stored at -80°C for testing at a later time.

5 159. Lymphocytes are removed from whole blood and diluted in media (e.g. RPMI 1640, DMEM, MEM, or EMEM) with 10% serum. PBL are then stained for antigen specific T cells using antigen specific MHC class I and MHC class II tetramers to be visualized via flow cytometry. Simultaneously or separately PBL are stained with surface markers to look at activation characteristics of the cells and other surface
10 phenotypes. Some examples of the antibodies used are CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45RA, CD45RO, CD62L (L-selectin), CD69 and Bcl-2. Additionally, PBL can be stained for the production of intracellular cytokines such as IFN- γ , IL-4, TNF- α , IL-2, and IL-10 following stimulation with antigen. Lymphocytes can also be used in an ELISPOT assay for cytokine production following stimulation
15 with specific antigen or CTL assay to assess killing activity.

 160. All of the stains and assays allow for the assessment of cell-mediated responses to the priming antigen. Tetramer staining allows for the enumeration of antigen specific T cells of a known specificity. This coupled with surface markers details the activation state of the antigen specific cells. Tetramer positive T cells
20 (CD8+/CD3+, CD3+, or CD3+/CD4+) possess various surface markers that reveal the activation state of the cells. By multiplying percentages obtained from the analysis of the staining by the number of cells, the exact number of cells exhibiting any characteristic can be obtained. Exposure to an antigen creates cells that express high levels of CD11a, and CD44. Once these levels are increased on a cell, they will not
25 decrease, and in conjunction with tetramer stains and other surface markers can lead to the identification of memory T cells. CD43 and CD69 both increase during activation, but decrease following clearance of the priming antigen. L-selecting will decrease on the surface of activated cells and increase over time to naïve levels. Bcl-2 also provides a measure of the activation level of cells as activated cells will have decreased Bcl-2
30 levels relative to naïve T cells indicating a susceptibility to apoptosis whereas memory cells will have increased levels of Bcl-2.

161. ELISPOT and intracellular cytokine stains allow the enumeration of cytokine secreting antigen-specific cells following stimulation. This can be paired with the number of tetramer positive cells to determine what portion, if not all, of the T cells are functional.

5 162. CTL assays determine the ability for antigen specific T cells to kill cells expressing the antigen. The assay reads on the cytolytic activity of T cells as determined by release of a chemiluminescent marker or radioactive material in labeled target cells.

10 163. Like the cell-mediated responses, humoral responses may be measured in harvested PBL. PBL maybe stained for anti-mouse IgG, CD45R (B220), PNA, and CD138 (syndecan-1) to look at the activation of B cells or the number of plasma cells. Plasma cells, the effector arm of the humoral immune system are syndecan-1+, and B220-. Memory B cells will be IgG+, B220+, and PNA+, the presence of PNA positive cells indicates transition through a germinal center reaction. Additionally,
15 PBL may be used to enumerate the number of antigen specific plasma cells on a plasma cell elispot or the number of antigen specific B cells on a memory B cell assay.

164. ELISA data can be combined with the humoral immunity data from the PBL to assess the effect of the immunization on the mouse.

20 165. Mice that have measurable responses can be boosted and responses monitored. However, direct challenge is necessary as it indicates the ability to mount a protective immune response. Mice that do not have an immune response or mice that have an immune response that but need to be boosted can receive second and third immunizations by the same or different route than the priming immunization over the next few months.

25 166. As mice cannot be infected with a lentivirus, monkeys (e.g., rhesus macaques) are used for challenge experiments. Monkeys can receive a similar construct (pHSV-SIV-gag) which produces similar levels of gag in vitro. Monkeys are inoculated with the pHSV-SIV-gag DNA, and after several weeks, they can be challenged with an infectious SIV. The animals can then be observed for protection
30 from infection or disease.

167. For a challenge, monkeys are given an infectious dose of an antigen (e.g. SIV) or stimulated in such a way as to induce a condition to be prevented. Challenged

monkeys can be assessed in the same way as immunized mice with additional assays used to look at antigen load following challenge and gross pathological and histological assessments made from tissue biopsy or necropsy.

168. Monkeys can also be given an infectious dose of an antigen (e.g. SIV) or stimulated in such a way as to create a condition to be treated. Once the condition is established, monkeys are given a therapeutic dose of the immunizing antigen and monitored as to the effects on the condition. A successful therapy will not necessarily clear the condition or infection, but may slow or stop progression. The same assays to those used for prophylactic immunization are used to characterize the responses

10 **Methods**

Flow cytometry

169. Cells are suspended at a concentration that is appropriate according to the antibody manufacturers instructions in FACS buffer (2% FCS (or BSA) in PBS (0.2% NaN₃ can also be added)). Antibodies (CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45R (B220), CD45RA, CD45RO, CD62L (L-selectin), CD69, CD138, PNA, anti-mouse IgG) are added to the cells according to the manufacturers instructions and incubated for 30min at 4C in the dark. Cells are washed 3X in FACS Buffer. 1 wash comprises centrifuging the cells at 800rpm for 3min to pellet the cells, removing the media, and resuspending the cells. After the third wash, cells are resuspended in 2% PFA in PBS. Staining is analyzed on a FACSCalibur flow cytometry instrument (Beckton-Dickenson) or other suitable cytometer.

Intracellular cytokine staining.

170. Cells are stimulated for 5hrs in the presence of antigen and BFA (meninsin may be substituted for BFA) at 37C (this stimulation is not needed for Bcl-2 staining). After the 5hr incubation, cells are centrifuged and the media removed and resuspended in FACS buffer (2% FCS (or BSA) in PBS (0.2% NaN₃ can also be added)). Antibodies (CD3, CD4, CD8, CD11a (LFA-1), CD43 (1B11), CD44, CD45R (B220), CD45RA, CD45RO, CD62L (L-selectin), CD69, CD138, PNA, anti-mouse IgG) are added to the cells according to the manufacturers instructions and incubated for 30min at 4C in the dark. Cells are washed 3X in FACS Buffer. 1 wash comprises centrifuging the cells at 800rpm for 3min to pellet the cells, removing the media, and resuspending the cells. After the third wash, cells are resuspended in cytofix/cytoperm

solution for 20min at 4C. Cells are then washed 3X in a permwash solution and resuspended at a concentration that is appropriate according to the antibody manufacturers instructions in permwash. Antibodies (IFN- γ , TNF- α , IL-2, IL-4, IL-10, and Bcl-2) are added to the cells according to the manufacturers instructions and

5 incubated for 30min at 4C in the dark. Cells are washed 3X in PermWash and 2X in FACS Buffer. After the second FACS Buffer wash, cells are resuspended in 2% PFA in PBS. Staining is analyzed on a FACSCalibur flow cytometry instrument (Beckton-Dickenson) or other suitable cytometer. Intracellular kits are commercial available through (Beckton-Dickenson).

10 ELISA

171. 96-well ELISA plates are coated with antigen overnight at 4C. Plates are blocked with a suitable blocking media containing 10% FCS for 1-2hrs at RT. Plates are resuspended in a known volume of an ELISA diluent (e.g., PBS+10% FCS) and serum is added to the plate and incubated at RT for 1.5hrs. Plates are washed with

15 PBS+0.1%Tween 3X and 100 μ l of antibodies (e.g., HRPO conjugated anti-mouse IgG) diluted 1:1000 in ELISA diluent is added to each well. Plates are incubated for 1.5hrs at RT and then washed 3x with PBS+0.1%Tween. Plates are then coated with 100 μ l of a chromagen substrate (e.g., o-phenyldiamine + 3% H₂O₂ in citrate buffer (Sodium citrate in H₂O pH=5) and incubated for 1hr in the dark. The reaction is stopped by

20 adding 100 μ l of 1N HCl. Plates are read in a ELISA plate reader.

Plasma cell ELISPOT

172. 96-well filter (ELISPOT) plates are coated with antigen overnight. Plates are washed 1X in PBS-0.1%Tween and 3X in PBS. Plate are blocked 1-2hrs with media + 10% FCS. After blocking, media is removed and 100 μ l of media are

25 added to each well. Effector cells (cells containing lymphocytes from an immunized animal (e.g., PBL, splenocytes, hepatocytes, and bone marrow) are added to the plate and serial dilutions are made. Plates are incubated for 5hrs at 37C. After the 5hr incubation, plates are washed 3X in PBS and 3X in PBS+0.1% Tween. Plates are then coated with 100 μ l PBS+0.1% Tween+1% FCS and biotinylated Ab at 1/100

30 concentration overnight at 4C. After incubation plates are washed 4X in PBS+0.1%Tween and incubated for 1hr at RT in PBS+0.1%Tween+1%FCS containing HRP-avidinD at 1/1000. Plates are washed 3X with PBS and a suitable chromagen

substrate is added (e.g., AEC). Plates are allowed to develop for 8-15min and the reaction is stopped with washing the plates with water. Spots are visualized and counted using a dissection microscope or ELISPOT plate reader.

Cytokine ELISPOT

- 5 173. The day prior to the assay coat each well with 50 μ l 10 μ g/ml purified anti-cytokine antibody (e.g., IFN- γ , TNF- α , IL-2, IL-4, IL-10). Incubate overnight at 4 °C. Wash plates 4 times with 200 μ l PBS. Block wells with 200 μ l 10%FCS complete medium, incubate at room temperature for at least 1 hour. Add effector cells to the top wells and make serial dilutions. Discard 100 μ l of cells from the last dilution. Add the
- 10 stimulating antigen and IL-2 to the wells. Incubate plates at 37 °C, 5% CO₂ for 16-40 hours (use level to ensure that plates stay even horizontally). Discard cells and wash 5 times with PBS-Tween). Add 100 μ l 2 μ g/ml biotinylated-anti-cytokine antibody (e.g., IFN- γ , TNF- α , IL-2, IL-4, IL-10) diluted in PBS-Tween. Incubate at room temperature for 2 hours or overnight at 4C. Wash plate 5 times with PBS-Tween, 200 μ l each wash.
- 15 Add 100 μ l 1:1000 Streptavidin -HRP, incubate 1 hour at room temperature. (avoid high background by not exceeding 1 hour incubation). Wash wells 5 times with PBS-Tween. Add 100 μ l chromagen substrate per well, incubate at room temperature for 10-20 minutes in dark (at 10 minutes, observe the color developed, if the color is not fully intense, incubate few more minutes and double check). Wash plates under
- 20 running tap water and air-dry plates (in hood with light turned-off). Count and record numbers of spots/well by ELISPOT plate reader or stereomicroscope (20x).

CTL

174. Direct ex-vivo cytolytic killing is measured in a 5hr ⁵¹Cr release assay. Briefly, target cells are labeled with 51Cr for 1hr at 37C. Following the 1hr incubation,
- 25 cells are centrifuged for 8min at 1200rpm to pellet the cells. The media is poured off and the pellet resuspended in fresh media. Cells are centrifuged and resuspended two additional times. After the third spin, cells are resuspended in an appropriate amount of media to attain the desired effector to target ration (e.g., a 50:1 effector: target ratio with effector cells at a concentration of 1x10⁷/ ml would require target cells to be at a
- 30 concentration of 2x10⁵/ml), and contacted with antigen. Effector cells (cells containing lymphocytes from an immunized animal (e.g., PBL, splenocytes, hepatocytes, and bone marrow) are contacted with the target cells for 5-6hrs at 37C. Cells are pelleted by

centrifugation and supernatants harvested. ^{51}Cr release is measured on an appropriate instrument. Percent killing is (experimental release-spontaneous release / maximum release – spontaneous release) x 100.

175. Secondary killing to measure memory cell responses to antigen can also
5 be measured. In this method, effector cells are incubated for 6 days in the presence of antigen stimulation and then used in a cytolytic assay as described above.

Example 3

176. In accordance with the methods described above the pHSV vector comprising an SIV-Gag-p17/p27 construct was made. This vector is useful to establish a model for the study of
10 the effectiveness of a particular treatment or prophylactic vaccine. For example, such a model can comprise a system to establish a foamy live viral vector for vaccine use. The pHSV with an SIV gag (p17,p27) insert has been tested in vitro and shown to express similar levels of SIV gag as the HIV gag engineered vector. This SIVgag live viral vector can be used to inoculate rhesus macaques
15 in order to determine protective immune responses that can develop following in vivo expression of the vector gene products including the SIV gag protein. Inoculated animals can then be challenged with wild type SIV to further determine any potential vaccine induced efficacy by studying primary (sterilizing immunity) and secondary (time to morbidity) end points.

CLAIMS

What is claimed:

1. A live replicating human spumavirus vector (pHSV) suitable for human use comprising an immunizing construct, wherein the immunizing construct partially replaces the *bet* gene.
2. The vector of claim 1, wherein the vector has the sequence of SEQ ID NO:1.
3. The vector of claim 1, further comprising a reporter gene.
4. The vector of claim 3, wherein the reporting gene is GFP.
5. The vector of claim 1, wherein the immunizing construct is an antigen encoding nucleic acid.
6. The vector of claim 5, wherein the antigen-encoding nucleic acid encodes a viral antigen.
7. The vector of claim 6, wherein the viral antigen is an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.
8. The vector of claim 7, wherein the viral antigen is HIV-GAG p17/p24.
9. The vector of claim 7, wherein the immunizing construct is SIV-GAG.
10. The vector of claim 5, wherein the antigen-encoding nucleic acid encodes a bacterial antigen.
11. The vector of claim 10, wherein the bacterial antigen is an antigen from a bacterium selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis*

strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

12. The vector of claim 5, wherein the peptide encoding nucleic acid encodes a parasitic antigen.

13. The vector of claim 12, wherein the parasitic antigen is an antigen from a parasite selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

14. The vector of claim 5, wherein the peptide encoding nucleic acid encodes a cancer antigen.

15. The vector of claim 14, wherein the cancer antigen is an antigen from a cancer selected from the group consisting of lymphomas (Hodgkins and non-Hodgkins), B cell lymphoma, T cell lymphoma, leukemias, myeloid leukemia, carcinomas, carcinomas of solid tissues, squamous cell carcinomas of mouth, throat, larynx, lung, head and neck, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, mycosis fungoides, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer,

neuroblastoma/glioblastoma, ovarian cancer, liver cancer, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, or pancreatic cancer.

16. The cancer antigen of claim 15, wherein the antigen is selected from the list of cancer antigen consisting of mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmell7, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

17. A cell comprising the vector of claim 1.

18. A method of detecting the expression of the vector of claim 1, comprising obtaining a sample containing the vector, contacting the sample with an antibody directed to antigen encoded by the immunizing construct, and detecting antigen bound to the antibody, the presence of bound antigen indicating expression of the vector.

19. The method of claim 18, wherein the sample is blood from a subject.

20. The method of claim 18, wherein the sample is tissue biopsy from a subject.

21. A method of treating a subject with a condition comprising administering to the subject the vector of claim 1.

22. The method of claim 21, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2,

Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma

virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

23. The method of claim 21, wherein the condition is associated with bacterial infection, and the immunizing construct encodes an antigen from a bacterium selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria monocytogenes*, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetii*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Haemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

24. The method of claim 21, wherein the condition is associated with parasitic infection, and the immunizing construct encodes an antigen from a parasite selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

25. The method of claim 21, wherein the condition is cancer, and the immunizing construct encodes a tumor antigen from the list consisting of human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast

cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, gp100/pmell17, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostate-specific antigen (PSA), HPV-16, MUM, alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7, Wilm's tumor antigen-1, telomerase, and melanoma gangliosides.

26. A method of preventing a condition in a subject comprising administering to the subject the vector of claim 1.

27. The method of claim 26, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papillomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency virus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

28. The method of claim 26, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of *M. tuberculosis*, *M. bovis*, *M. bovis* strain BCG, BCG substrains, *M. avium*, *M. intracellulare*, *M. africanum*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. avium* subspecies *paratuberculosis*, *Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis*, *Pasteurella haemolytica*, *Pasteurella multocida*, other *Pasteurella* species, *Actinobacillus pleuropneumoniae*, *Listeria*

monocytogenes, *Listeria ivanovii*, *Brucella abortus*, other *Brucella* species, *Cowdria ruminantium*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Coxiella burnetti*, other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Escherichia coli*, *Vibrio cholerae*, *Campylobacter* species, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Haemophilus influenzae*, *Haemophilus ducreyi*, other *Hemophilus* species, *Clostridium tetani*, other *Clostridium* species, *Yersinia enterocolitica*, and other *Yersinia* species.

29. The method of claim 28, wherein the condition is associated with viral infection, and the immunizing construct encodes an antigen from a virus selected from the group consisting of *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, other *Plasmodium* species., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* species., *Schistosoma mansoni*, other *Schistosoma* species., and *Entamoeba histolytica*.

30. A method of making a model for use in screening for substances effective in treating or preventing a disease associated with an immunizing construct, comprising administering the vector of claim 1 to an animal capable of manifesting a characteristic of the immunizing construct.

31. A model made in accordance with the method of claim 30.

32. A method of screening for a substance effective in treating a disease associated with an immunizing construct comprising: a) administering the substance to the model of claim 31; and b) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

33. A method of screening for an immunizing construct effective in preventing a disease associated with an immunizing construct comprising: a) administering the vector of claim 1 to a subject; b) subjecting the subject to treatment that will induce the disease or characteristic of the disease; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an

improvement in the course of the disease in the presence of the substance identifies a substance that is effective in preventing the disease.

34. A method of screening for an immunizing construct effective in treating a disease effective in treating a disease associated with an immunizing construct comprising: a) subjecting the subject to treatment that will induce the disease or characteristic of the disease; b) administering the vector of claim 1 to a subject; and c) assaying for an change in the course of the disease as compared to an the course of the disease in a control subject; an improvement in the course of the disease in the presence of the substance identifies a substance that is effective in treating the disease.

35. The method of claims 21-34, wherein the subject is a cell.

36. The method of claims 21-34, wherein the subject is a mouse.

37. The method of claims 21-34, wherein the subject is a monkey.

38. The method of claims 21-34, wherein the subject is a human

1/8

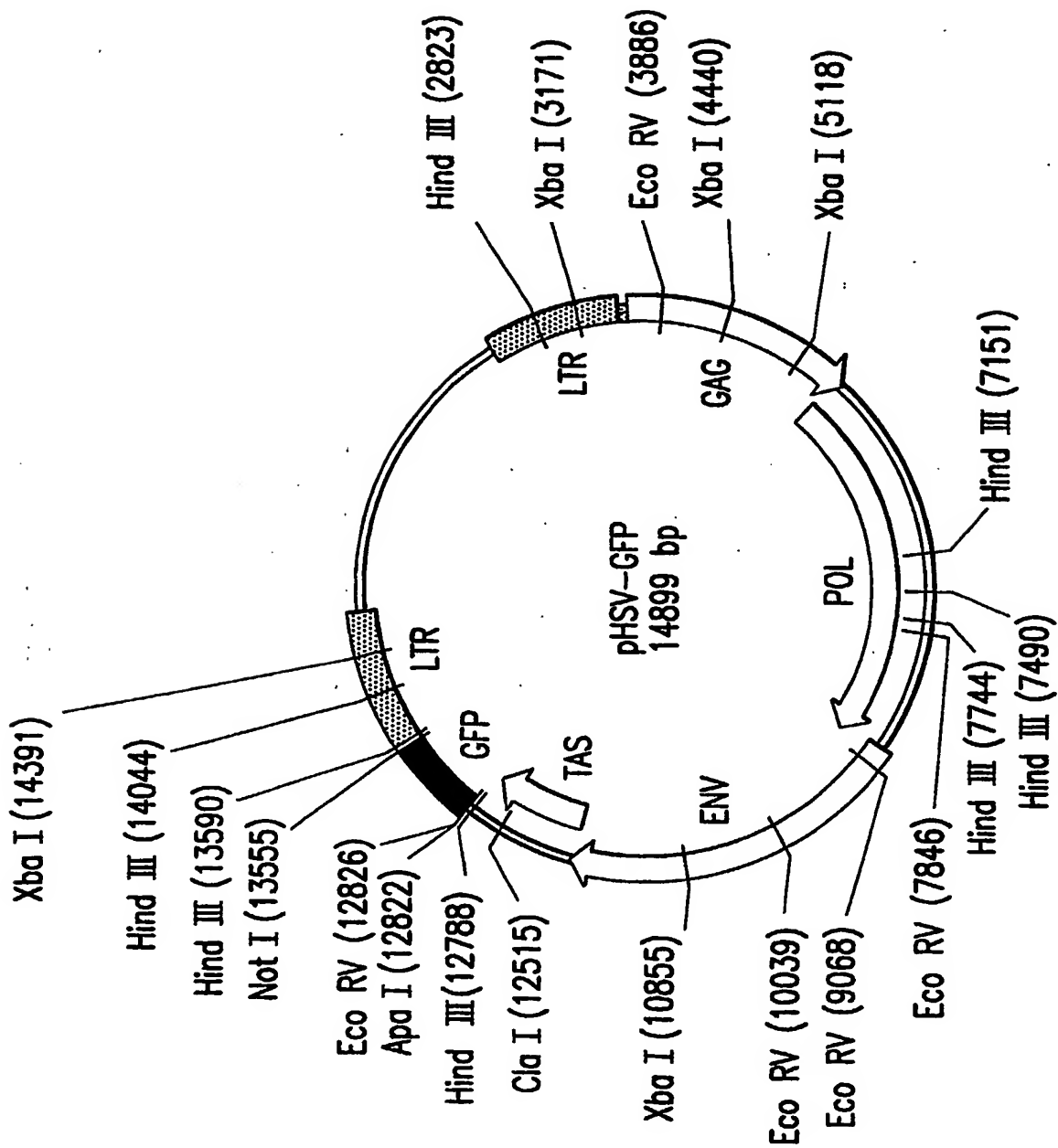


FIG.1

2/8

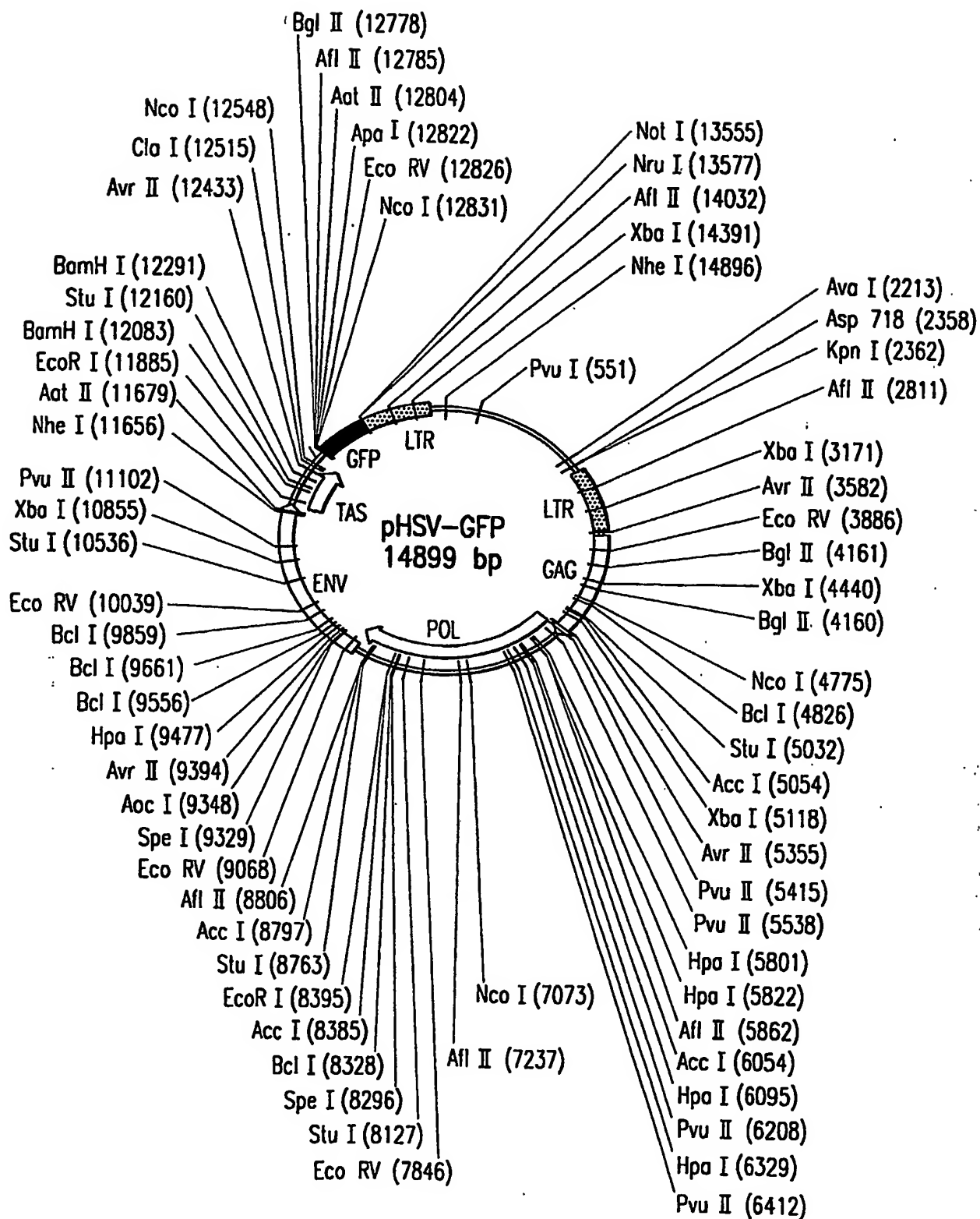


FIG.2

SUBSTITUTE SHEET (RULE 26)

3/8

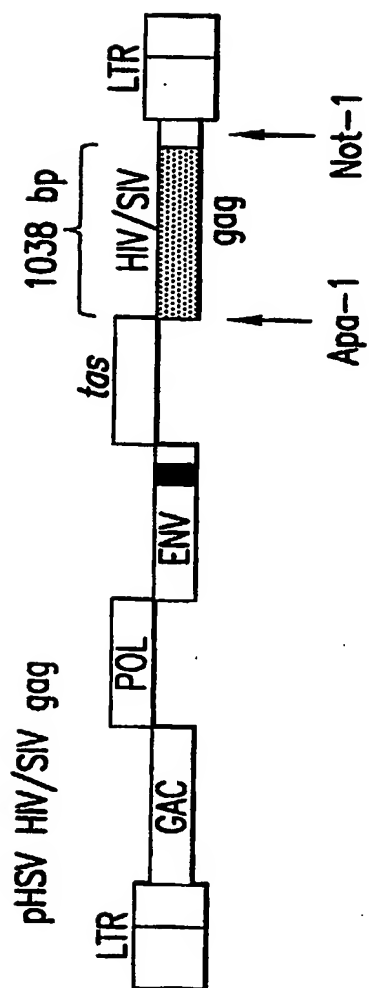


FIG.3

4/8

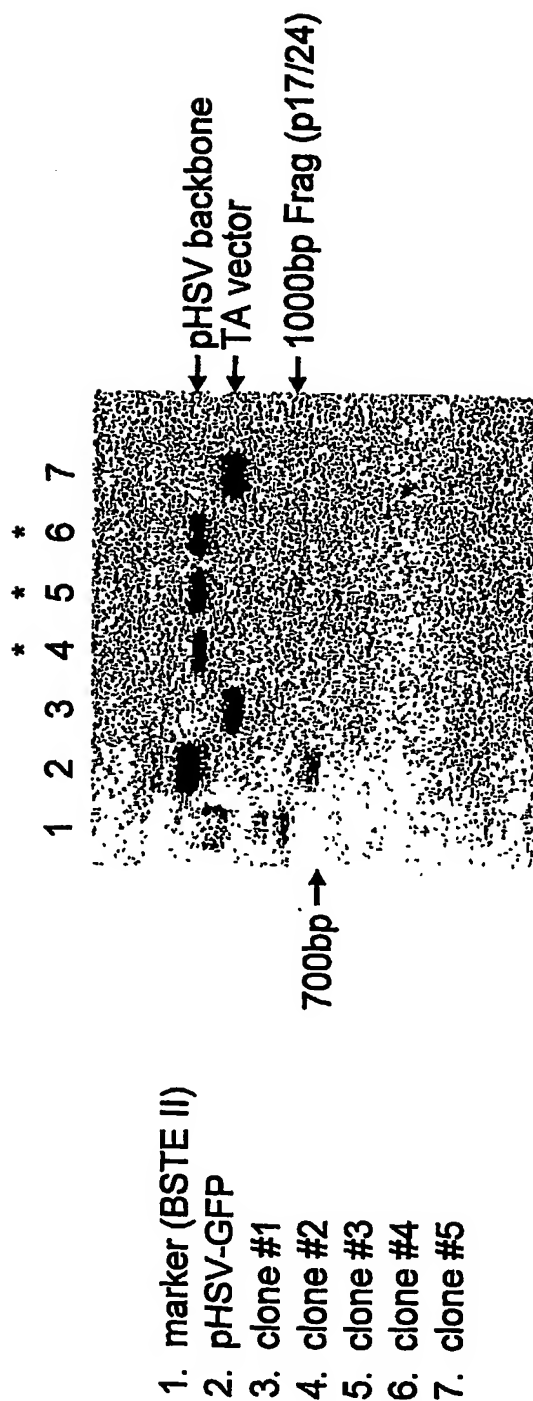


FIG.4

BEST AVAILABLE COPY

5/8

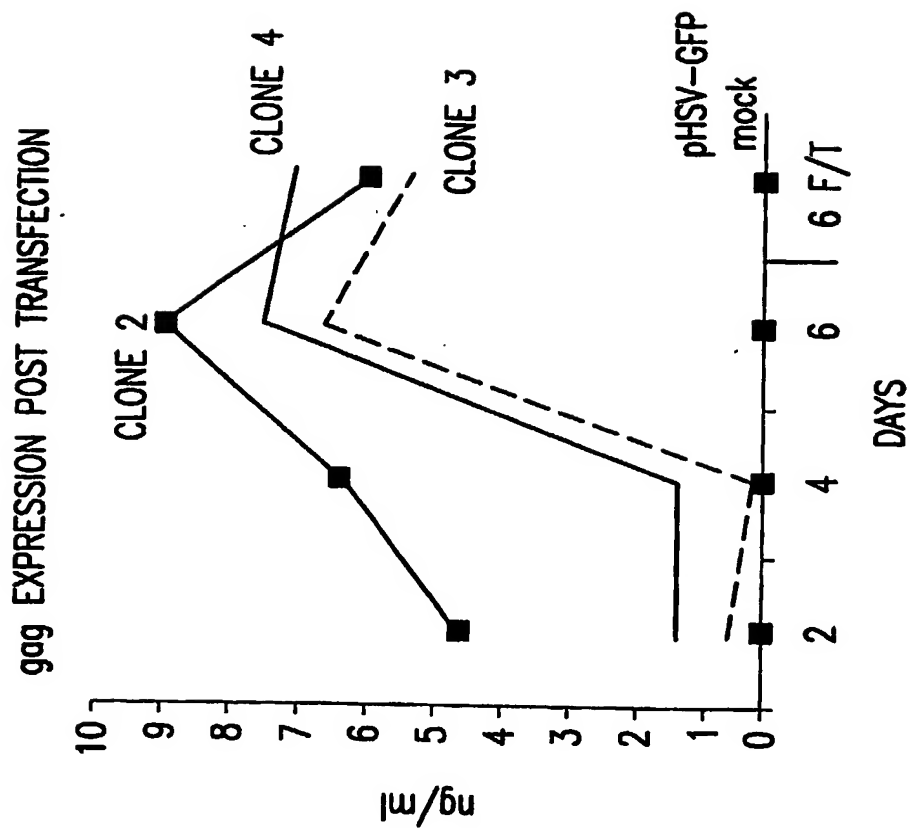


FIG.5

6/8

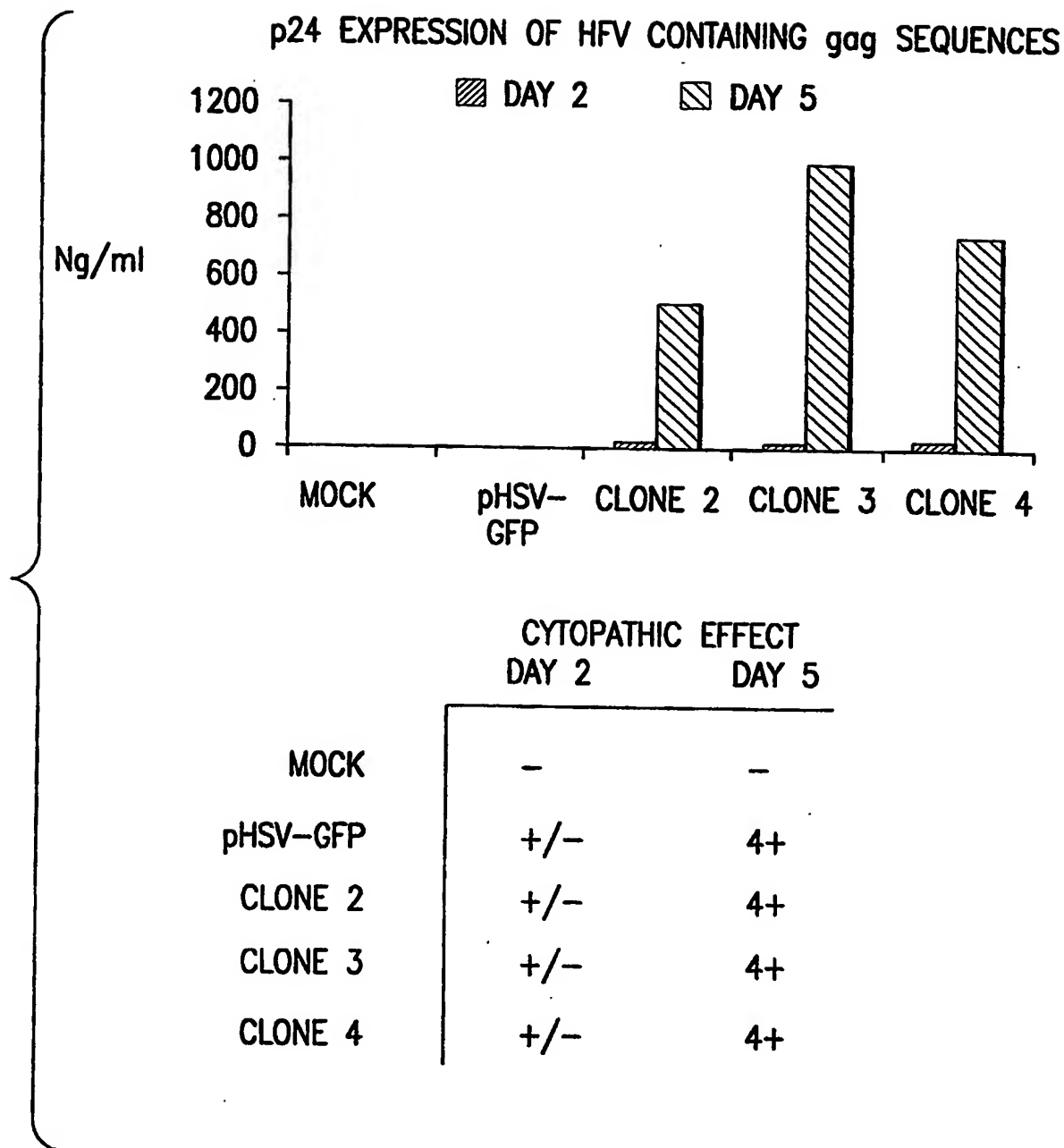


FIG.6

7/8

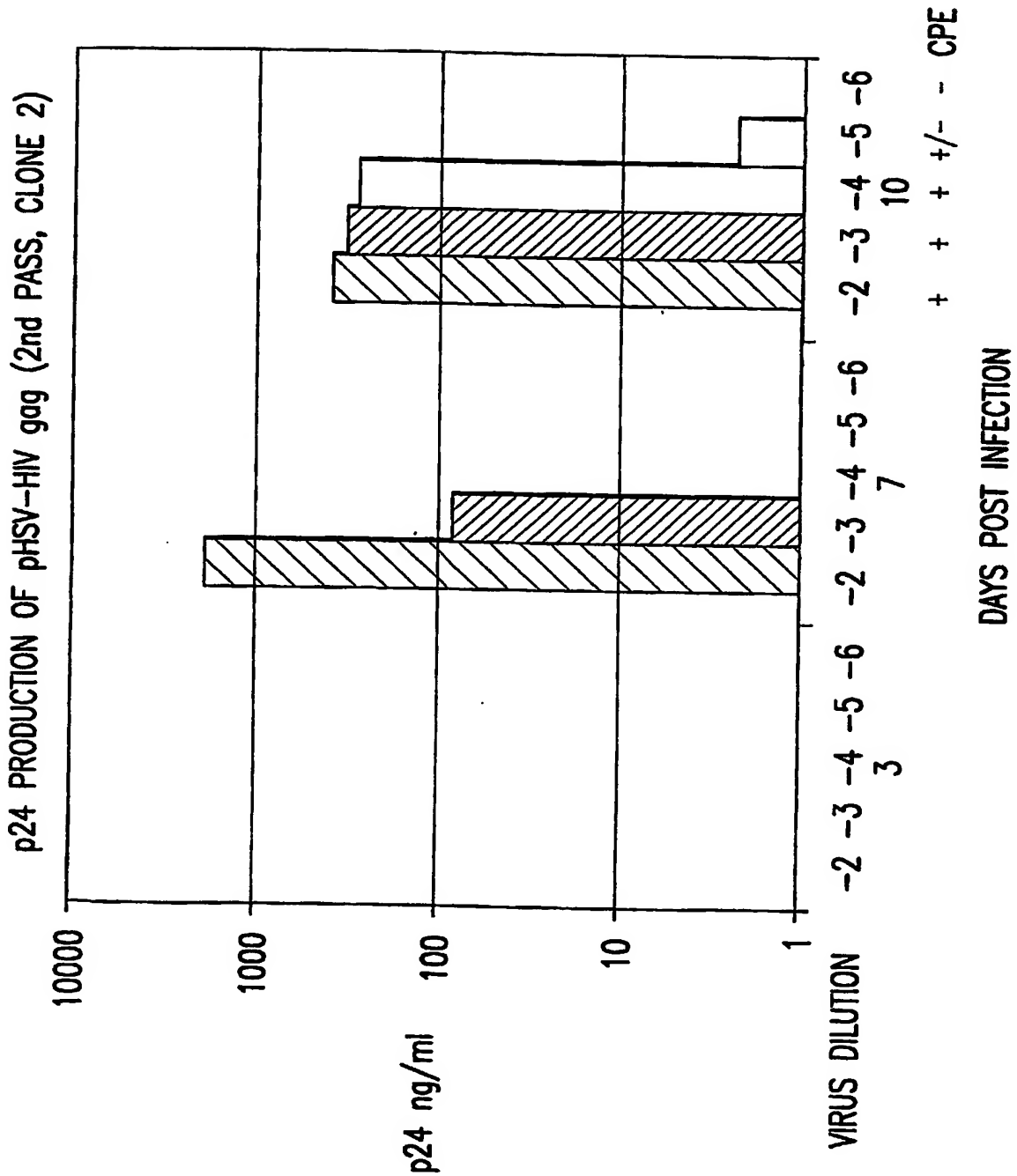


FIG. 7

8/8

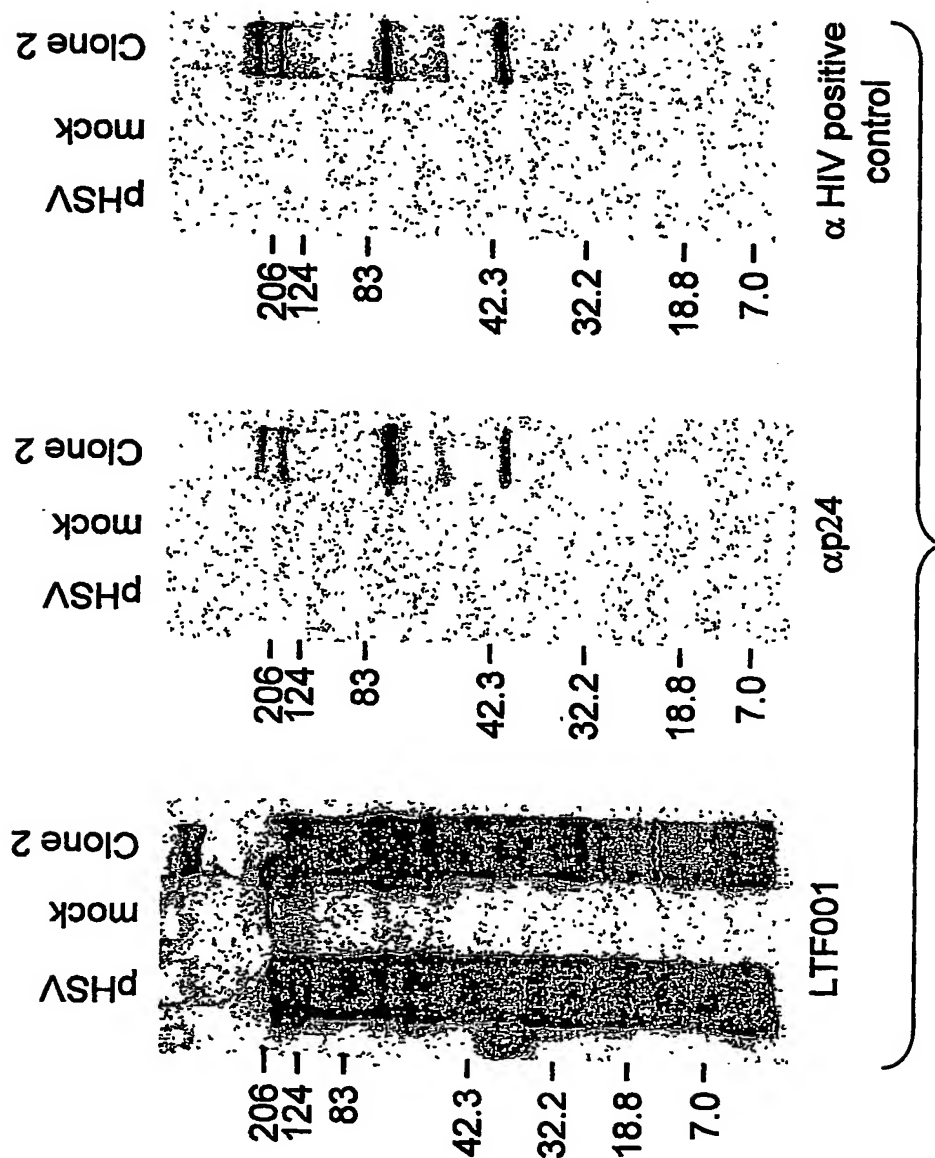


FIG. 8

BEST AVAILABLE COPY

SEQ. ID NO. I

```
1  CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT
   GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA
-----
51  TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCTGATA
   AAGATTTATG TAAGTTTATA CATAGGCGAG TACTCTGTTA TTGGGACTAT
-----
101 AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC
   TTACGAAGTT ATTATAACTT TTTCTTCTC ATACTCATAA GTTGTAAGG
-----
151 GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT
   CACAGCGGGA ATAAGGGAAA AAACGCCGTA AAACGGAAGG ACAAAAACGA
-----
201 CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC
   GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG
-----
251 ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA
   TGCTCACCCA ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACCTC
-----
301 GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
   CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC
-----
351 CTATGTGGCG CGGTATTATC CCGTGTGAC GCCGGGCAAG AGCAACTCGG
   GATACACCGC GCCATAATAG GGCACAACTG CGGCCCGTTC TCGTTGAGCC
-----
401 TCGCCGCATA CACTATTCTC AGAATGACTT GGTGAGTAC TCACCAGTCA
   AGCGGCTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT
-----
451 CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT
   GTCTTTTCGT AGAATGCCTA CCGTACTGTC ATTCTCTTAA TACGTCACGA
-----
501 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT
   CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA
-----
551 CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG
   GCCTCCTGGC TTCCTCGATT GCGCAAAAAA CGTGTGTGAC CCCCTAGTAC
-----
601 TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATAACAAAC
   ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG
-----
651 GACGAGCGTG ACACCACGAT GCCTGCAGCA ATGGCAACAA CGTTGCGCAA
   CTGCTCGCAC TGTGGTGCTA CGGACGTCGT TACCGTTGTT GCAACGCGTT
-----
701 ACTATTAACT GCGGAACCTAC TTA CTCTAGC TTCCCGGCAA CAATTAATAG
   TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC
-----
751 ACTGGATGGA GCGGATAAAA GTTG CAGGAC CACTTCTGCG CTCGGCCCTT
   TGACCTACCT CCGCCTATTT CAACGTCCTG GTGAAGACGC GAGCCGGGAA
-----
801 CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC
   GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG
-----
851 TCGCGGTATC ATTG CAGCAC TGGGGCCAGA TCCATTCGGG AGGGCATAGC
   AGCGCCATAG TAACGTCGTG ACCCGGTCT AGGTAAGCCC TCCCGTATCG
-----
901 ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGGTAAGCCC
   TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACCATTCGGG
```



```

951  TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA
     AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT
-----
1001  ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT
     TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA TTCGTAACCA
-----
1051  AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAACTTT
     TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA
-----
1101  CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT
     GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC TATTAGAGTA
-----
1151  GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG
     CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC
-----
1201  TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC
     ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAGA CGCGCATTAG
-----
1251  TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC
     ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTGCGCCACC AAACAAACGG
-----
1301  GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAAGTGGC TTCAGCAGAG
     CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG AAGTCGTCTC
-----
1351  CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC
     GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG
-----
1401  TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT
     AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG ATTAGGACAA
-----
1451  ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT
     TGGTCACCGA CGACGGTCAC CGCTATTAG CACAGAATGG CCCAACCTGA
-----
1501  CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTGCGGCTG AACGGGGGGT
     GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC TTGCCCCCCA
-----
1551  TCGTGACACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA
     AGCAGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT
-----
1601  CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG
     GGATGTCGCA CTCGATACTC TTTCGCGGTG CGAAGGGCTT CCTCTTTCC
-----
1651  CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG
     GCCTGTCCAT AGGCCATTCT CCGTCCCAGC CTTGTCTCTCT CGCGTGCTCC
-----
1701  GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG
     CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC AGCCCCAAGC
-----
1751  CCACCTCTGA CTTGAGCGTC GATTTTGTG ATGCTCGTCA GGGGGGCGGA
     GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCTT
-----
1801  GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT
     CGGATACCTT TTGCGGTCTG TTGCGCCGGA AAAATGCCAA GGACCGGAAA
-----
1851  TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT
     ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA
-----

```

```

2851  CAATCAACAA AACAATGATG ATGTAATCAT AAGGAAGTAG TTTAAAATAG
      GTTAGTTGTT TTGTTACTAC TACATTAGTA TTCCTTCATC AAATTTTATC
-----
2901  GTTAATAAGT TTATTAGTTA TATAGAAAAT AATATAGGAT AAAGTATAAG
      CAATTATTCA AATAATCAAT ATATCTTTTA TTATATCCTA TTTCATATTC
-----
2951  GATTAAGGTA TGAGGTGTGT GGCTCAACAC GTAGGGTGAC AAGAAAATCT
      CTAATTCCAT ACTCCACACA CCGAGTTGTG CATCCCACTG TTCTTTTAGA
-----
3001  ACTGTAATAG GACACAACAC CTCTAAAGTT GCCCGTGCGA AGGTGAAGTG
      TGACATTATC CTGTGTTGTG GAGATTTCAA CGGGCACCCT TCCACTTCAC
-----
3051  AGATCGAATC TTCCTTAAC GCAGACAGCT TTTTATCCAC TAGGGATAAT
      TCTAGCTTAG AAAGGAATTG CGTCTGTCGA AAAATAGGTG ATCCCTATTA
-----
3101  GTTTTAAGGA ATACTATAGT AATAGATTGA TAGTTTTAAC AATGATGGAA
      CAAAATTCCT TATGATATCA TTATCTAACT ATCAAAATTG TTACTACCTT
-----
3151  ATAGTATATA AGGATAGTTT CTAGATTGTA CGGGAGCTCT TCACTACTCG
      TATCATATAT TCCTATCAAA GATCTAACAT GCCCTCGAGA AGTGATGAGC
-----
3201  CTGCGTCGAG AGTGTCGAG ACTCTCCAGG TTTGGTAAGA AATATTTTAT
      GACGCAGCTC TCACATGCTC TGAGAGGTCC AAACCATTCT TTATAAATA
-----
3251  ATTGTTATAA TGTTACTATG ATCCATTAAC ACTCTGCTTA TAGATTGTAA
      TAACAATATT ACAATGATAC TAGGTAATTG TGAGACGAAT ATCTAACATT
-----
3301  GGGTGATTGC AATGCCTTCT GCATAAAACT TTGGTTTTCT TGTTAATCAA
      CCCACTAACG TTACGAAAGA CGTATTTTGA AACCAAAAGA ACAATTAGTT
-----
3351  TAAACCGACT TGATTGAGTA ACCTACTCAT ATATTATTGT CTCTTTTATA
      ATTTGGCTGA ACTAAGCTCT TGGATGAGTA TATAATAACA GAGAAAATAT
-----
3401  CTTTATTAAG TAAAAGGATT TGTATATTAG CTTTGCTAAG GGAGACATCT
      GAAATAATTC ATTTTCCTAA ACATATAATC GGAACGATTC CCTCTGTAGA
-----
3451  AGTGATATAA GTGTGAACTA CACTTATCTT AAATGATGTA ACTCCTTAGG
      TCACTATATT CACACTTGAT GTGAATAGAA TTTACTACAT TGAGGAATCC
-----
3501  ATAATCAATA TACAAAATTC CATGACAATT GGCGCCCAAC GTGGGGCTCG
      TATTAGTTAT ATGTTTTAAG GTACTGTTAA CCGCGGGTTG CACCCCGAGC
-----
3551  AATATAAGTC GGGTTTATTT GTAAATTATC CCTAGGGACC TCCGAGCATA
      TTATAATTCAG CCCAAATAAA CATTTAATAG GGATCCCTGG AGGCTCGTAT
-----
3601  GCGGGAGGCA TATAAAAGCC AATAGACAAT GGCTTCAGGA AGTAATGTTG
      CGCCCTCCGT ATATTTTCGG TTATCTGTTA CCGAAGTCCT TCATTACAAC
-----
3651  AAGAATATGA ACTTGATGTT GAAGCTCTGG TTGTAATTTT AAGAGATAGA
      TTCTTATACT TGAAC TACAA CTTTCGAGACC AACATTAAAA TTCTCTATCT
-----
3701  AATATACCAA GAAATCCTTT ACATGGAGAA GTTATAGGTC TTCGCCTTAC
      TTATATGGTT CTTTAGGAAA TGTACCTCTT CAATATCCAG AAGCGGAATG
-----
3751  TGAAGGATGG TGGGGACAAA TTGAGAGATT TCAGATGGTA CGTCTAATAT
      ACTTCCTACC ACCCCTGTTT AACTCTCTAA AGTCTACCAT GCAGATTATA
-----

```

3801 TACAAGATGA TGATAATGAA CCTTTACAGA GACCTAGATA TGAGGTAATA
ATGTTCTACT ACTATTACTT GGAAATGTCT CTGGATCTAT ACTCCATTAT

3851 CAACGAGCTG TAAACCCTCA TACAATGTTT ATGATATCAG GACCATTAGC
GTTGCTCGAC ATTTGGGAGT ATGTTACAAA TACTATAGTC CTGGTAATCG

3901 TGAACCTCAA TTAGCCTTTC AGGATTTAGA TTTACCTGAA GGTCCATTGA
ACTTGAAGTT AATCGGAAAG TCCTAAATCT AAATGGACTT CCAGGTAAC

3951 GGT TTGGTCC ATTGGCAAAT GGACATTATG TTCAAGGAGA TCCTTATAGT
CCAAACCAGG TAACCGTTTA CCTGTAATAC AAGTTCCTCT AGGAATATCA

4001 AGTTCTTACA GACCAGTAAC AATGGCCGAA ACAGCCCAA TGACTAGAGA
TCAAGAATGT CTGGTCATTG TTACCGGCTT TGTCGGGTTT ACTGATCTCT

4051 TGAACCTGAA GATGTTCTTA ATACTCAAAG TGAAATAGAA ATTCAAATGA
ACTTGACCTT CTACAAGAAT TATGAGTTTC ACTTTATCTT TAAGTTTACT

4101 TAAATTTATT GGAGTTGTAT GAAGTTGAAA CTAGAGCTCT TAGAAGACAA
ATTTAAATAA CCTCAACATA CTTCAACTTT GATCTCGAGA ATCTTCTGTT

4151 TTAGCTGAGA GATCTAGTAC AGGGCAAGGA GGAATATCCC CAGGAGCTCC
AATCGACTCT CTAGATCATG TCCCGTTCCT CTTTATAGGG GTCCTCGAGG

4201 TCGTTCCTGA CCACCAGTAA GCAGCTTCTC AGGGTTACCA AGTTTGCCCT
AGCAAGAGCT GGTGGTCATT CGTCGAAGAG TCCCAATGGT TCAAACGGGA

4251 CTATACCTGG GATTCATCCC AGGGCACCTT CACCTCCAAG GGCAACTTCT
GATATGGACC CTAAGTAGGG TCCCGTGAA GTGGAGGTTT CCGTTGAAGA

4301 ACTCCCGGAA ATATTCCTTG GAGTTTAGGA GATGATAGCC CACCTTCATC
TGAGGGCCTT TATAAGGAAC CTCAAATCCT CTACTATCGG GTGGAAGTAG

4351 TAGTTTTCTT GGACCCTCTC AACCTCGTGT TTCTTTCCAT CCGGGAAATC
ATCAAAGGA CCTGGGAGAG TTGGAGCACA AAGAAAGGTA GGCCCTTTAG

4401 CTTTTGTTGA AGAAGAAGGT CATAGACCTA GATCCCAGTC TAGAGAAAGG
GAAAACAACT TCTTCTTCCA GTATCTGGAT CTAGGGTCAG ATCTCTTTCC

4451 AGAAGAGAAA TTCTTCCTGC TCCTGTACCG TCAGCACCTC CTATGATTCA
TCTTCTCTTT AAGAAGGACG AGGACATGGC AGTCGTGGAG GATACTAAGT

4501 GTATATACCA GTACCACTC CACCACCGAT TGGCACGGTT ATACCTATTC
CATATATGGT CATGGTGGAG GTGGTGGCTA ACCGTGCCAA TATGGATAAG

4551 AGCATATCAG ATCTGTAACCT GGAGAGCCTC CTAGAAACCC AAGAGAAATA
TCGTATAGTC TAGACATTGA CCTCTCGGAG GATCTTTGGG TTCTCTTTAT

4601 CCAATTTGGC TAGGACGAAA TGCTCCTGCT ATAGATGGAG TGTTCCCTGT
GGTTAAACCG ATCCTGCTTT ACGAGGACGA TATCTACCTC ACAAGGGACA

4651 TACAACACCG GATCTAAGAT GCAGAATAAT TAATGCTATA CTAGGAGGAA
ATGTTGTGGC CTAGATTCTA CGTCTTATTA ATTACGATAT GATCCTCCTT

4701 ATATTGGGCT ATCATTAACC CCTGGAGACT GTTTAACATG GGAATCAGCA
TATAACCCGA TAGTAATTGG GGACCTCTGA CAAATTGTAC CCTGAGTCGT

4751 GTAGCCACCT TATTTATTAG AACCCATGGA ACTTTTCCAA TGCATCAGCT
CATCGGTGGA ATAAATAATC TTGGGTACCT TGAAAAGGTT ACGTAGTCGA

4801 TGGAAATGTA ATAAAAGGCA TAGTTGATCA AGAAGGAGTG GCAACAGCAT
ACCTTTACAT TATTTTCCGT ATCAACTAGT TCTTCCTCAC CGTTGTCGTA

4851 ATACTTTGGG AATGATGCTT TCTGGACAAA ATTATCAATT AGTTTCTGGA
TATGAAACCC TTACTACGAA AGACCTGTTT TAATAGTTAA TCAAAGACCT

4901 ATAATTAGAG GATATTTGCC TGGACAAGCT GTAGTAACTG CATTACAACA
TATTAATCTC CTATAACGG ACCTGTTCTGA CATCATTGAC GTAATGTTGT

4951 GCGTTTAGAC CAAGAAATAG ATAATCAAAC AAGAGCAGAG ACTTTTATTC
CGCAAATCTG GTTCTTTATC TATTAGTTTG TTCTCGTCTC TGAAAATAAG

5001 AACATCTAAA TGCTGTATAT GAAATTTTAG GCCTTAATGC CAGAGGACAA
TTGTAGATTT ACGACATATA CTTTAAATC CGGAATTACG GTCTCCTGTT

5051 AGTATACGTG CTTCAGTGAC TCCTCAACCC CGACCATCCA GAGGTAGAGG
TCATATGCAC GAAGTCACTG AGGAGTTGGG GCTGGTAGGT CTCCATCTCC

5101 TCGAGGTCAA AATACTTCTA GACCCTCTCA AGGACCAGCT AATAGCGGGC
AGCTCCAGTT TTATGAAGAT CTGGGAGAGT TCCTGGTCTGA TTATCGCCCC

5151 GGGGACGACA GCGCCCTGCT TCTGGTCAAA GCAACAGAGG ATCTAGTACT
CCCTGCTGTG CGCGGGACGA AGACCAGTTT CGTTGTCTCC TAGATCATGA

5201 CAGAATCAAA ATCAAGATAA TTTAAATCAA GGAGGATATA ATCTTCGACC
GTCTTAGTTT TAGTTCTATT AAATTTAGTT CCTCTATAT TAGAAGCTGG

5251 CCGTACTTAC CAACCTCAAA GGTACGGAGG AGGACGTGGA CGAAGATGGA
GGCATGAATG GTTGGAGTTT CCATGCCTCC TCCTGCACCT GCTTCTACCT

5301 ACGATAATAC TAACAATCAA GAGTCCAGAC CATCAGATCA AGGTTCTCAA
TGCTATTATG ATTGTTAGTT CTCAGGTCTG GTAGTCTAGT TCCAAGAGTT

5351 ACTCCTAGGC CAAATCAAGC AGGCTCTGGG GTGCGTGGCA ATCAGTCACA
TGAGGATCCG GTTTAGTTCTG TCCGAGACCC CACGCACCGT TAGTCAGTGT

5401 AACTCCCAGA CCAGCTGCTG GTCGCGGAGG AAGAGGTAAC CACAACCGAA
TTGAGGCTCT GGTGACGAC CAGCGCCTCC TTCTCCATTG GTGTTGGCTT

5451 ACCAACGATC ATCCGGTGCT GGTGACTCAC GCGCTGTCAA TACCGTGACA
TGGTTGCTAG TAGGCCACGA CCACTGAGTG CGCGACAGTT ATGGCACTGT

5501 CAGAGTGCCA CGTCCTCCAC AGATGAATCC TCTTCAGCTG TTACAGCCGC
GTCTCACGGT GCAGGAGGTG TCTACTTAGG AGAAGTCGAC AATGTCGGCG

5551 TTCCGGCGGA GATCAAAGGG ACTAAATTGT TAGCCCACTG GGATTCAGGG
AAGGCCGCT CTAGTTTCCC TGATTTAACA ATCGGGTGAC CCTAAGTCCC

5601 GCAACAATAA CTTGTATTCC TGAAAGTTT TTAGAAGATG AACAACTAT
CGTTGTTATT GAACATAAGG ACTTTCAAAA AATCTTCTAC TTGTTGGATA

5651 TAAAAAGACT TTAATAAAAA CAATTCATGG AGAAAAACAA CAAAATGTTT
ATTTTCTGA AATTATTTTT GTTAAGTACC TCTTTTGTGTT GTTTTACAAA

```

5701  ATTATGTAAC  CTTTAAAGTT  AAAGGAAGAA  AAGTGGAAGC  AGAAGTGATA
      TAATACATTG  GAAATTTCAA  TTTCTTCTT  TTCACCTTCG  TCTTCACTAT
-----
5751  GCTTCTCCTT  ATGAGTATAT  TTTGCTGTCG  CCAACAGATG  TTCCTTGGTT
      CGAAGAGGAA  TACTCATATA  AAACGACAGC  GGTGTCTTAC  AAGGAACCAA
-----
5801  AACACAGCAA  CCACTTCAGT  TAACAATTTT  AGTTCCTCTT  CAAGAATATC
      TTGTGTCGTT  GGTGAAGTCA  ATTGTTAAAA  TCAAGGAGAA  GTTCTTATAG
-----
5851  AAGAGAAAAA  CTTAAGTAAG  ACTGCTCTTC  CAGAAGATCA  AAAACAACAA
      TTCTCTTTTA  GAATTCATTG  TGACGAGAAG  GTCTTCTAGT  TTTTGTGTGT
-----
5901  TTAAAAACCT  TGTTTGTCAA  GTATGACAAT  CTATGGCAAC  ATTGGGAAAA
      AATTTTTGGA  ACAACAGTT  CATACTGTTA  GATACCGTTG  TAACCCTTTT
-----
5951  TCAAGTCGGG  CATAGAAAAA  TTAGGCCACA  TAATATAGCA  ACTGGTGATT
      AGTTCAGCCC  GTATCTTTTT  AATCCGGTGT  ATTATATCGT  TGACCACTAA
-----
6001  ATCCTCCTCG  CCCTCAAAAA  CAATATCCTA  TTAATCCTAA  GGCAAAGCCT
      TAGGAGGAGC  GGGAGTTTTT  GTTATAGGAT  AATTAGGATT  CCGTTTCGGA
-----
6051  AGTATACAAA  TTGTAATAGA  TGACTTATTG  AAACAAGGGG  TGTTAACGCC
      TCATATGTTT  AACATTATCT  ACTGAATAAC  TTTGTTCCTC  ACAATTGCGG
-----
6101  TCAAAATAGT  ACAATGAATA  CACCAGTGTA  TCCTGTTTCT  AAACCAGATG
      AGTTTTATCA  TGTTACTTAT  GTGGTCACAT  AGGACAAGGA  TTTGGTCTAC
-----
6151  GAAGGTGGAG  AATGGTATTA  GATTATAGAG  AAGTAAATAA  AACTATTCCA
      CTTCCACCTC  TTACCATAAT  CTAATATCTC  TTCATTTATT  TTGATAAGGT
-----
6201  TTAACAGCTG  CCAAAAACCA  ACACTCTGCT  GGTATTTTAG  CTACTATTGT
      AATTGTCGAC  GGGTTTTGGT  TGTGAGACGA  CCATAAAATC  GATGATAACA
-----
6251  TAGACAAAAA  TATAAAACTA  CCTTAGATTT  AGCTAATGGA  TTTTGGGCTC
      ATCTGTTTTT  ATATTTTGAT  GGAATCTAAA  TCGATTACCT  AAAACCCGAG
-----
6301  ATCCTATTAC  ACCAGAATCT  TATTGGTTAA  CAGCATTAC  CTGGCAAGGT
      TAGGATAATG  TGGTCTTAGA  ATAACCAATT  GTCGTAAATG  GACCGTTCCA
-----
6351  AAACAGTATT  GTTGGACAG  TCTTCCTCAA  GGATTTTAA  ATAGTCCAGC
      TTTGTCATAA  CAACCTGTGC  AGAAGGAGTT  CCTAAAAATT  TATCAGGTGC
-----
6401  ATTGTTTACA  GCTGATGTAG  TAGATTTACT  AAAAGAAATC  CCTAATGTAC
      TAACAAATGT  CGACTACATC  ATCTAAATGA  TTTTCTTTAG  GGATTACATG
-----
6451  AAGTGATATG  TGATGATATA  TATTTAAGCC  ATGATGATCC  TAAAGAGCAT
      TTCACATACA  ACTACTATAT  ATAAATTCGG  TACTACTAGG  ATTTCTCGTA
-----
6501  GTTCAACAAT  TAGAAAAAGT  GTTTCAAATT  TTACTACAGG  CAGGATATGT
      CAAGTTGTTA  ATCTTTTTCA  CAAAGTTTAA  AATGATGTCC  GTCCTATACA
-----
6551  AGTATCTTTG  AAAAAATCAG  AAATTGGTCA  AAAAACTGTA  GAATTTTTAG
      TCATAGAAAC  TTTTTTAGTC  TTTAACCAGT  TTTTGTGACAT  CTTAAAAATC
-----
6601  GATTTAATAT  TACTAAAGAA  GGTCGTGGCC  TAACAGACAC  TTTTAAACAA
      CTAAATTATA  ATGATTTCTT  CCAGCAACGG  ATTGTCTGTG  AAAATTTTGT
-----

```

6651 AAAGTGTAA ATATTACTCC TCCAAAAGAC TTAAAGCAAT TACAAAGCAT
TTTGACAATT TATAATGAGG AGGTTTTCTG AATTTCGTTA ATGTTTCGTA

6701 ATTAGGATTG TTAAATTTTG CTAGAAATTT TATACCTAAT TTTGCTGAAC
TAATCCTAAC AATTTAAAAC GATCTTTAAA ATATGGATTA AAACGACTTG

6751 TGGTACAACC ATTATACAAT TTAATAGCCT CAGCAAAGG CAAATATATT
ACCATGTTGG TAATATGTTA AATTATCGGA GTCGTTTTCC GTTTATATAA

6801 GAGTGGTCTG AAGAAAATAC TAAACAATTA AATATGGTAA TAGAAGCATT
CTCACCAGAC TTCTTTTATG ATTTGTAAAT TTATACCATT ATCTTCGTAA

6851 AAACACTGCC TCTAATTTAG AAGAAAGGTT ACCAGAACAG AGACTGGTAA
TTTGTGACGG AGATTAAATC TTCTTTCCAA TGGTCTTGTC TCTGACCATT

6901 TTAAAGTCAA TACTTCTCCA TCAGCAGGAT ATGTAAGATA TTATAATGAG
AATTTCAAGT ATGAAGAGGT AGTCGTCCTA TACATTCTAT AATATTACTC

6951 ACTGGTAAAA AGCCTATTAT GTACCTAAAT TATGTGTTTT CCAAAGCAGA
TGACCATTTT TCGGATAATA CATGGATTTA ATACACAAAA GGTTCGTCT

7001 ATTAAATTTT TCTATGTTAG AAAAATATT AACTACAATG CACAAAGCCT
TAATTTTAAA AGATACAATC TTTTGTGATA TTGATGTTAC GTGTTTCGGA

7051 TAATTAAGGC TATGGATTTG GCCATGGGAC AAGAAATATT AGTTTATAGT
ATTAATTCGG ATACCTAAAC CGGTACCCTG TTCTTTATAA TCAAATATCA

7101 CCCATTGTAT CTATGACTAA AATACAAAAA ACTCCACTAC CAGAAAGAAA
GGGTAACATA GATACTGATT TTATGTTTTT TGAGGTGATG GTCTTTCTTT

7151 AGCTTTACCC ATTAGATGGA TAACATGGAT GACTTATTTA GAAGATCCAA
TCGAAATGGG TAATCTACCT ATTGTACCTA CTGAATAAAT CTTCTAGGTT

7201 GAATCCAATT TCATTATGAT AAAACCTTAC CAGAACTTAA GCATATTCCA
CTTAGGTTAA AGTAATACTA TTTTGAATG GTCTTGAATT CGTATAAGGT

7251 GATGTATATA CATCTAGTCA GTCTCCTGTT AAACATCCTT CTCAATATGA
CTACATATAT GTAGATCAGT CAGAGGACAA TTTGTAGGAA GAGTTATACT

7301 AGGAGTGTTT TATACTGATG GCTCGGCCAT CAAAAGTCCT GATCCTACAA
TCCTCACAAA ATATGACTAC CGAGCCGGTA GTTTTCAGGA CTAGGATGTT

7351 AAAGCAATAA TGCTGGCATG GGAATAGTAC ATGCCACATA CAAACCTGAA
TTTCGTTATT ACGACCGTAC CCTTATCATG TACGGTGTAT GTTTGGACTT

7401 TATCAAGTTT TGAATCAATG GTCAATACCA CTAGGTAATC ATACTGCTCA
ATAGTTCAAA ACTTAGTTAC CAGTTATGGT GATCCATTAG TATGACGAGT

7451 GATGGCTGAA ATAGCTGCAG TTGAATTTGC CTGTAAAAAA GCTTTAAAAA
CTACCGACTT TATCGACGTC AACTTAAACG GACATTTTTT CGAAATTTTT

7501 TACCTGGTCC TGTATTAGTT ATAAGTGATA GTTTCTATGT AGCAGAAAGT
ATGGACCAGG ACATAATCAA TATTGACTAT CAAAGATACA TCGTCTTTCA

7551 GCTAATAAAG AATTACCATA CTGGAAATCT AATGGGTTTG TTAATAATAA
CGATTATTTT TTAATGGTAT GACCTTTAGA TTACCCAAAC AATTATTATT

7601 GAAAAAGCCT. CTTAAACATA TCTCCAAATG GAAATCTATT GCTGAGTGTT
CTTTTCGGA GAATTTGTAT AGAGGTTTAC CTTTAGATAA CGACTCACAA

7651 TATCTATGAA ACCAGACATT ACTATTCAAC ATGAAAAAGG CATCAGCCTA
ATAGATACTT TGGTCTGTAA TGATAAGTTG TACTTTTTCC GTAGTCGGAT

7701 CAAATACCAG TATTCATACT GAAAGGCAAT GCCCTAGCAG ATAAGCTTGC
GTTTATGGTC ATAAGTATGA CTTTCCGTTA CGGGATCGTC TATTCGAACG

7751 CACCCAAGGA AGTTATGTGG TTAATTGTAA TACCAAAAAA CCAAACCTGG
GTGGGTTTCT TCAATACACC AATTAACATT ATGGTTTTTT GGTTCGGACC

7801 ATGCAGAGTT GGATCAATTA TTACAGGGTC ATTATATAAA AGGATATCCC
TACGTCTCAA CCTAGTTAAT AATGTCCCAG TAATATATTT TCCTATAGGG

7851 AAACAATATA CATATTTTTT AGAAGATGGC AAAGTAAAAG TTTCCAGACC
TTTGTTATAT GTATAAAAAA TCTTCTACCG TTTCATTTTC AAAGGTCTGG

7901 TGAAGGGGTT AAAATTATTC CCCCTCAGTC AGACAGACAA AAAATTGTGC
ACTTCCCCAA TTTAATAAAG GGGGAGTCAG TCTGTCTGTT TTTTAACACG

7951 TTCAAGCCCA CAATTTGGCT CACACCGGAC GTGAAGCCAC TCTTTTAAAA
AAGTTCGGGT GTTAAACCGA GTGTGGCCTG CACTTCGGTG AGAAAATTTT

8001 ATTGCCAACC TTTATTGGTG GCCAAATATG AGAAAGGATG TGGTTAAACA
TAACGGTTGG AAATAACCAC CGGTTTATAC TCTTTCCTAC ACCAATTTGT

8051 ACTAGGACGC TGTCAACAGT GTTTAATCAC AAATGCTTCC AACAAAGCCT
TGATCCTGCG ACAGTTGTCA CAAATTAGTG TTTACGAAGG TTGTTTCGGA

8101 CTGGTCCTAT TCTAAGACCA GATAGGCCTC AAAAACCTTT TGATAAATTC
GACCAGGATA AGATTCTGGT CTATCCGGAG TTTTGGAAA ACTATTTAAG

8151 TTTATTGAAT ATATTGGACC TTTGCCACCT TCACAGGGAT ACCTATATGT
AAATAACTGA TATAACCTGG AAACGGTGGA AGTGTCCCTA TGGATATACA

8201 ATTAGTAGTT GTTGATGGAA TGACAGGATT CACTTGGTTA TACCCCACTA
TAATCATCAA CAACTACCTT ACTGTCCTAA GTGAACCAAT ATGGGGTGAT

8251 AGGCTCCTTC TACTAGCGCA ACTGTTAAAT CTCTCAATGT ACTCACTAGT
TCCGAGGAAG ATGATCGCGT TGACAATTTA GAGAGTTACA TGAGTGATCA

8301 ATTGCAATTC CAAAGGTGAT TCACTCTGAT CAAGGTGCAG CATTCACTTC
TAACGTTAAG GTTTCCACTA AGTGAGACTA GTTCCACGTC GTAAGTGAAG

8351 TTCAACCTTT GCTGAATGGG CAAAGGAAAAG AGGTATACAT TTGGAATTCA
AAGTTGGAAA CGACTTACCC GTTTCTTTC TCCATATGTA AACCTTAAGT

8401 GTACTCCTTA TCACCCCAA AGTGGTAGTA AGGTGGAAA GAAAAATAGT
CATGAGGAAT AGTGGGGGTT TCACCATCAT TCCACCTTTC CTTTTTATCA

8451 GATATAAAAC GACTTTTAACT TAACTGCTA GTAGGAAGAC CCACAAAGTG
CTATATTTTG CTGAAAATTG ATTTGACGAT CATCCTTCTG GGTGTTTCAC

8501 GTATGACCTA TTGCCTGTTG TACAACCTGC TTAAACAAC ACCTATAGCC
CATACTGGAT AACGGACAAC ATGTTGAACG AAATTTGTTG TGGATATCGG

```

8551 CTGTATTAAA ATATACTCCA CATCAACTCT TATTTGGTAT AGATTCAAAT
      GACATAATTT TATATGAGGT GTAGTTGAGA ATAAACCATA TCTAAGTTTA
-----
8601 ACTCCATTTC CAAATCAAGA TACACTTGAC TTGACCAGAG AAGAAGAAGT
      TGAGGTAAAC GTTTAGTTCT ATGTGAACTG AACTGGTCTC TTCTTCTTGA
-----
8651 TTCTCTTTTA CAGGAAATTC GTACTTCTTT ATACCATCCA TCCACCCCTC
      AAGAGAAAAT GTCCTTTAAG CATGAAGAAA TATGGTAGGT AGGTGGGGAG
-----
8701 CAGCCTCCTC TCGTTCCTGG TCTCCTGTTG TTGGCCAATT GGTCCAGGAG
      GTCGGAGGAG AGCAAGGACC AGAGGACAAC AACCGGTAA CCAGGTCCTC
-----
8751 AGGGTGGCTA GGCCTGCTTC TTTGAGACCT CGTTGGCATA AACCGTCTAC
      TCCCACCGAT CCGGACGAAG AAACCTGGA GCAACCGTAT TTGGCAGATG
-----
8801 TGTACTTAAG GTGTTGAATC CAAGGACTGT TGTATTTTTG GACCATCTTG
      ACATGAATTC CACAACCTAG GTTCCTGACA ACAATAAAAC CTGGTAGAAC
-----
8851 GCAACAACAG AACTGTAAGT ATAGATAATT TAAAACCTAC TTCTCATCAG
      CGTTGTTGTC TTGACATTCA TATCTATTAA ATTTTGATG AAGAGTAGTC
-----
8901 AATGGCACCA CCAATGACAC TGCAACAATG GATCATTTGG AAAAAATGA
      TTACCGTGGT GGTTACTGTG ACGTTGTTAC CTAGTAAACC TTTTTTACT
-----
8951 AATAAGCGCA TGAGGCACTT CAAAATACAA CAACTGTGAC TGAACAGCAG
      TATTCGCGT ACTCCGTGAA GTTTTATGTT GTTGACACTG ACTTGTCGTC
-----
9001 AAGGAACAAA TTATACTGGA CATTCAAAAT GAAGAAGTAC AACCAACTAG
      TTCCTTGTTT AATATGACCT GTAAGTTTTA CTTCTTCATG TTGGTTGATC
-----
9051 GAGAGATAAA TTTAGATATC TGCTTTATAC TTGTTGTGCT ACTAGCTCAA
      CTCTCTATTT AAATCTATAG ACGAAATATG AACAACACGA TGATCGAGTT
-----
9101 GAGTATTGGC CTGGATGTTT TTAGTTTGTA TATTGTTAAT CATTGTTTTG
      CTCATAACCG GACCTACAAA AATCAAACAT ATAACAATTA GTAACAAAAC
-----
9151 GTTTCATGCT TTGTGACTAT ATCCAGAATA CAATGGAATA AGGATATTCA
      CAAAGTACGA AACACTGATA TAGGTCTTAT GTTACCTTAT TCCTATAAGT
-----
9201 GGTATTAGGA CCTGTAATAG ACTGGAATGT TACTCAAAGA GCTGTTTATC
      CCATAATCCT GGACATTATC TGACCTTACA ATGAGTTTCT CGACAAATAG
-----
9251 AACCCTTACA GACTAGAAGG ATTGCACGTT CCCTTAGAAT GCAGCATCCT
      TTGGGAATGT CTGATCTTCC TAACGTGCAA GGAATCTTA CGTCGTAGGA
-----
9301 GTTCCAAAAT ATGTGGAGGT AAATATGACT AGTATTCCAC AAGGTGTATA
      CAAGGTTTTA TACACCTCCA TTTATACTGA TCATAAGGTG TTCCACATAT
-----
9351 CTATGAACCC CATCCGAAC CCATAGTGGT GAAGGAGAGG GTCCTAGGTC
      GATACTTGGG GTAGGCCTTG GGTATCACCA CTCCTCTCC CAGGATCCAG
-----
9401 TTTCTCAAAT TCTGATGATT AATTCAGAAA ACATTGCTAA TAATGCTAAT
      AAAGAGTTTA AGACTACTAA TTAAGTCTTT TGTAACGATT ATTACGATTA
-----
9451 TTGACACAAG AAGTAAAGAA GTTGTTAACT GAAATGGTTA ATGAAGAAAT
      AACTGTGTTC TTCATTTCTT CAACAATTGA CTTTACCAAT TACTTCTTTA
-----

```



```

9501  GCAAAGTTTG TCAGATGTAA TGATTGACTT TGAAATTCCT TTAGGAGACC
      CGTTTCAAAC AGTCTACATT ACTAACTGAA ACTTTAAGGA AATCCTCTGG
-----
9551  CTCGTGATCA AGAACAATAT ATACATAGAA AATGCTATCA AGAATTTGCA
      GAGCACTAGT TCTTGTTATA TATGTATCTT TTACGATAGT TCTTAAACGT
-----
9601  AATTGTTATT TAGTAAAATA TAAAGAACCC AAACCGTGGC CTAAGGAGGG
      TTAACAATAA ATCATTTTAT ATTTCTTGGG TTTGGCACCG GATTCTCTCC
-----
9651  CCTTATAGCT GATCAATGCC CATTACCAGG TTACCATGCT GGATTAACCT
      GGAATATCGA CTAGTTACGG GTAATGGTCC AATGGTACGA CCTAATTGGA
-----
9701  ATAATAGACA GTCTATTTGG GATTACTATA TTAAAGTGGA GAGTATTAGA
      TATTATCTGT CAGATAAACC CTAATGATAT AATTTACCT CTCATAATCT
-----
9751  CCTGCAAATT GGACAACAAA GAGTAAATAT GGACAAGCTA GACTAGGAAG
      GGACGTTTAA CCGTTGTTTT CTCATTTATA CCTGTTTCGAT CTGATCCTTC
-----
9801  TTTTTATATT CCTAGCAGCC TGAGACAAAT CAATGTTAGT CATGTACTAT
      AAAAATATAA GGATCGTCGG ACTCTGTTTA GTTACAATCA GTACATGATA
-----
9851  TCTGTAGTGA TCAATTATAT TCTAAATGGT ATAATATAGA AAATACCATA
      AGACATCACT AGTTAATATA AGATTTACCA TATTATATCT TTTATGGTAT
-----
9901  GAACAAAACG AGCGGTTTCT GCTTAATAAA CTAAATAACC TTACATCTGU
      CTTGTTTTGC TCGCCAAAGA CGAATTATTT GATTTATTGG AATGTAGACC
-----
9951  AACCTCAGTA TTGAAGAAAA GAGCTCTTCC GAAGGATTGG AGTTCTCAAG
      TTGGAGTCAT AACTTCTTTT CTCGAGAAGG CTTCTAACC TCAAGAGTTC
-----
10001 GTAAAAATGC TCTGTTTAGA GAAATCAATG TGTTAGATAT CTGCAGTAAA
      CATTTTTACG AGACAAATCT CTTTAGTTAC ACAATCTATA GACGTCATTT
-----
10051 CCTGAATCTG TAATACTATT GAATACTTCA TACTATTCCT TCTCTTTATG
      GGACTTAGAC ATTATGATAA CTTATGAAGT ATGATAAGGA AGAGAAATAC
-----
10101 GGAAGGAGAT TGTAATTTTA CTAAAGATAT GATTTCTCAG TTGGTTCCAG
      CCTTCCTCTA ACATTAAAAAT GATTTCTATA CTAAAGAGTC AACCAAGGTC
-----
10151 AATGTGATGG ATTTTATAAC AATTCTAAGT GGATGCATAT GCATCCATAT
      TTACACTACC TAAATATATTG TTAAGATTCA CCTACGTATA CGTAGGTATA
-----
10201 GCTTGTAGAT TCTGGAGAAG TAAGAAGAAT GAAAAAGAAG AAACATAAATG
      CGAACATCTA AGACCTCTTC ATTCTTCTTA CTTTTTCTTC TTTGATTTAC
-----
10251 TAGAGATGGG GAAACTAAGA GATGTCTGTA TTATCCTTTA TGGGACAGTC
      ATCTCTACCC CTTTGATTCT CTACAGACAT AATAGGAAAT ACCCTGTCAG
-----
10301 CCGAATCTAC ATATGATTTT GGTATTTTAG CATAACAAAA GAATTTTCCT
      GGCTTAGATG TATACTAAAA CCAATAAATC GTATGGTTTT CTTAAAAGGA
-----
10351 TCCCCTATCT GTATAGAACA ACAGAAAATT AGAGATCAAG ATTATGAAGT
      AGGGGATAGA CATATCTTGT TGTCTTTTAA TCTCTAGTTC TAATACTTCA
-----
10401 CTATTCCTTG TATCAAGAAC GCAAAATAGC TTCTAAAGCA TATGGAATTG
      GATAAGAAAC ATAGTTCTTG CGTTTTATCG AAGATTTCTG ATACCTTAAC
-----

```

```

10451 ATACAGTTTT ATTCTCTCTA AAGAATTTTC TTAATTATAC AGGAACTCCT
      TATGTCAAAA TAAGAGAGAT TTCTTAAAAG AATTAATATG TCCTTGAGGA
-----
10501 GTAAATGAAA TGCCTAATGC AAGAGCTTTT GTAGGCCTAA TAGATCCCAA
      CATTACTTTT ACGGATTACG TTCTCGAAAA CATCCGGATT ATCTAGGGTT
-----
10551 GTTTCCTCCT TCCTATCCCA ATGTTACTAG GGAACATTAT ACTTCCTGTA
      CAAAGGAGGA AGGATAGGGT TACAATGATC CCTTGTAATA TGAAGGACAT
-----
10601 ATAATAGGAA AAGAAGAAGT GTTGATAATA ACTATGCTAA GTTAAGGTCT
      TATTATCCTT TTCTTCTTCA CAACTATTAT TGATACGATT CAATTCCAGA
-----
10651 ATGGGGTATG CACTTACAGG AGCAGTGCAA ACCTTATCTC AAATATCAGA
      TACCCCATAC GTGAATGTCC TCGTACGTT TGGAAATAGAG TTTATAGTCT
-----
10701 TATTAATGAT GAAAACCTAC AGCAAGGAAT ATATTTATTA AGGGATCATG
      ATAATTACTA CTTTGAATG TCGTTCCTTA TATAAATAAT TCCCTAGTAC
-----
10751 TAATAACCTT AATGGAAGCT ACATTGCATG ATATATCTGT TATGGAAGGA
      ATTATTGGAA TTACCTTCGA TGTAACGTAC TATATAGACA ATACCTTCCT
-----
10801 ATGTTTGCTG TACAACATTT GCATACACAT TTGAATCATT TGAAGACAAT
      TACAAACGAC ATGTTGTAAA CGTATGTGTA AACTTAGTAA ACTTCTGTTA
-----
10851 GCTTCTAGAA AGAAGAATAG ACTGGACCTA TATGTCTAGT ACTTGGCTAC
      CGAAGATCTT TCTTCTTATC TGACCTGGAT ATACAGATCA TGAACCGATG
-----
10901 AACAACAATT ACAGAAATCT GATGATGAGA TGAAAGTAAT AAAGAGAATT
      TTGTTGTTAA TGTCTTTAGA CTACTACTCT ACTTTCATTA TTTCTCTTAA
-----
10951 GCTAGAAGTT TGGTATATTA TGTAAACAA ACCCATAGTT CTCCCACAGC
      CGATCTTCAA ACCATATAAT ACAATTTGTT TGGGTATCAA GAGGGTGTGC
-----
11001 TACAGCCTGG GAGATTGGAT TATATTATGA ATTGGTTATA CCTAAACATA
      ATGTCGGACC CTCTAACCTA ATATAATACT TAACCAATAT GGATTTGTAT
-----
11051 TTTACTTGAA TAATTGGAAT GTTGTCATA TAGGTCACCT AGTTAAATCA
      AAATGAACTT ATTAACCTTA CAACAGTTAT ATCCAGTGAA TCAATTTAGT
-----
11101 GCTGGACAAT TGAATCATGT AACTATAGCT CATCCTTATG AAATAATCAA
      CGACCTGTTA ACTGAGTACA TTGATATCGA GTAGGAATAC TTTATTAGTT
-----
11151 TAAGGAATGT GTAGAGACTA TATATCTGCA TCTTGAGGAC TGCACAAGAC
      ATTCCTTACA CATCTCTGAT ATATAGACGT AGAACTCCTG ACGTGTCTGT
-----
11201 AAGATTATGT CATATGTGAT GTGGTAAAGA TAGTGCAGCC TTGTGGCAAT
      TTCTAATACA GTATACACTA CACCATTCTT ATCAGTCGG AACACCGTTA
-----
11251 AGCTCAGACA CGAGTGATTG TCCTGTCTGG GCTGAAGCTG TAAAAGAACC
      TCGAGTCTGT GCTCACTAAC AGGACAGACC CGACTTCGAC ATTTTCTTGG
-----
11301 ATTTGTGCAA GTCAATCCTC TGAAAAACGG AAGTTATCTG GTTTTGSCAA
      TAAACACGTT CAGTTAGGAG ACTTTTGGCC TTCAATAGAC CAAAACCGTT
-----
11351 GTTCCACAGA CTGTCAGATC CCACCATATG TTCCTAGCAT CGTGACTGTT
      CAAGGTGTCT GACAGTCTAG GGTGGTATAC AAGGATCGTA GCACTGACAA
-----

```

```

11401  AATGAAACAA CGTCATGCTT TGGACTGGAC TTTAAAAGGC CACTGGTTGC
      TTACTTTTGT GCAGTACGAA ACCTGACCTG AAATTTTCCG GTGACCAACG
-----
11451  GGAAGAAAGA TTGAGCTTTG AGCCACGACT GCCAAATCTA CAACTAAGAT
      CCTTCTTTCT AACTCGAAAC TCGGTGCTGA CGGTTTAGAT GTTGATTCTA
-----
11501  TACCACATTT GGTGGAATT ATTGCAAAAA TCAAAGGGAT AAAAATAGAA
      ATGGTGTAAG CCAACCTTAA TAACGTTTTT AGTTTCCCTA TTTTATCTT
-----
11551  GTCACATCCT CTGGAGAAAG TATAAAGAG CAGATTGAAA GAGCAAAAGC
      CAGTGTAGGA GACCTCTTTC ATATTTTCTC GTCTAACTTT CTCGTTTTTC
-----
11601  TGAGCTCCTT CGACTGGACA TTCACGAGGG AGATACTCCT GCCTGGATAC
      ACTCGAGGAA GCTGACCTGT AAGTGCTCCC TCTATGAGGA CGGACCTATG
-----
11651  AACAGCTAGC TGCAGCAACA AAGGACGTCT GGCCAGCAGC AGCTTCTGCT
      TTGTCGATCG ACGTCGTTGT TTCCTGCAGA CCGGTCGTCG TCGAAGACGA
-----
11701  CTACAAGGAA TTGGTAACTT TTTATCTGGG ACTGCCCAAG GAATATTTGG
      GATGTTTCTT AACCATTGAA AAATAGACCC TGACGGGTTT CTTATAAACC
-----
11751  AACTGCCTTT AGTCTCTTGG GATACTTAAA GCCTATCCTA ATAGGAGTAG
      TTGACGGAAA TCAGAGAACC CTATGAATTT CGGATAGGAT TATCCTCATC
-----
11801  GGGTCATTCT CTTGGTTATT CTTATATTTA AGATTGTATC ATGGATTCTT
      CCCAGTAAGA GAACCAATAA GAATATAAAT TCTAACATAG TACCTAAGGA
-----
11851  ACGAAAAAGA AGAATCAGTA GCCTCCACCT CTGGAATTCA AGACCTGCAG
      TGCTTTTTTCT TCTTAGTCAT CGGAGGTGGA GACCTTAAGT TCTGGACGTC
-----
11901  ACTCTGAGTG AGCTTGTTGG TCCTGAAAAT GCCGGAGAGG GAGAGCTGAC
      TGAGACTCAC TCGAACAACC AGGACTTTTA CGGCCTCTCC CTCTCGACTG
-----
11951  TATTGCTGAG GAACCTGAAG AAAATCCTCG ACGCCCCAGA CGATATACTA
      ATAACGACTC CTTGGACTTC TTTTAGGAGC TGCGGGGTCT GCTATATGAT
-----
12001  AAAGAGAAGT CAAATGTGTG TCTTATCATG CATATAAAGA AATTGAGGAC
      TTTCTCTTCA GTTTACACAC AGAATAGTAC GTATATTTCT TTAACCTCTG
-----
12051  AAACATCCTC AACATATTAA ACTGCAGGAT TGGATCCCCA CACCAGAGGA
      TTTGTAGGAG TTGTATAATT TGACGTCCTA ACCTAGGGGT GTGGTCTCCT
-----
12101  AATGAGTAAG TCACTCTGTA AAAGACTTAT TTTATGTGGA TTGTATAGTG
      TTAATCATTC AGTGAGACAT TTTCTGAATA AAATACACCT AACATATCAC
-----
12151  CAGAAAAGGC CTCAGAGATT TTAAGGATGC CTTTACAGT TTCTTGGGAA
      GTCTTTTCCG GAGTCTCTAA AATTCCTACG GAAAATGTCA AAGAACCCTT
-----
12201  CAATCAGATA CTGACCCTGA CTGTTTTATT GTAAGCTATA CATGTATATT
      GTTAGTCTAT GACTGGGACT GACAAAATAA CATTGATAT GTACATATAA
-----
12251  TTGTGATGCT GTAATACATG ATCCCATGCC CATAAGATGG GATCCTGAAG
      AACACTACGA CATTATGTAC TAGGGTACCG GTATTCTACC CTAGGACTTC
-----
12301  TTGGAATTTG GGTAAATAT AAACCCCTCA GAGGAATTGT TGGATCTGCT
      AACCTTAAAC CCATTTTATA TTTGGGGAGT CTCCTTAACA ACCTAGACGA
-----

```

```

12351  GTGTTTATTA  TGCATAAACA  TCAAAGAAAC  TGTTCCTCTTG  TTAAACCTTC
      CACAAATAAT  ACGTATTTGT  AGTTTCTTTG  ACAAGAGAAC  AATTTGGAAG
-----
12401  TACCAGTCGC  TCAGAAGGTC  CAAAACCAAG  ACCTAGGCAC  GATCCTGTCC
      ATGGTCAGCG  AGTCTTCCAG  GTTTGGGTTT  TGGATCCGTG  CTAGGACAGG
-----
12451  TTCGATGTGA  CATGTTTGAA  AAGCATCACA  AGCCTCGGCA  GAAACGACCC
      AAGCTACACT  GTACAAACTT  TTCGTAGTGT  TCGGAGCCGT  CTTTGCTGGG
-----
12501  AGGAGACGAT  CCATCGATAA  TGAGTCATGT  GCTTCCAGTA  GTGACACCAT
      TCCTCTGCTA  GGTAGCTATT  ACTCAGTACA  CGAAGGTCAT  CACTGTGGTA
-----
12551  GGCCAATGAG  CCAGGATCAC  TATGCACCAA  CCCTCTTTGG  AATCCTGGAC
      CCGGTTACTC  GGTCCTAGTG  ATACGTGGTT  GGGAGAAACC  TTAGGACCTG
-----
12601  CGCTACTATC  AGGGCTACTT  GAAGAGTCCA  GCAACCTACC  AAAGTTGGAA
      GCGATGATAG  TCCCGATGAA  CTTCTCAGGT  CGTTGGATGG  TTTGAACCTT
-----
12651  GTTCACATGT  CAGGTGGACC  CTTCTGGGAA  GAGGTTTATG  GGGACTCAAT
      CAAGTGATCA  GTCCACCTGG  GAAGACCCTT  CTCCAAATAC  CCCTGAGTTA
-----
12701  TTTGGGTCCC  CCCTCTGGGT  CAGGTGAACA  TTCAGTTTTA  TAAGAATTAT
      AAACCCAGGG  GGGAGACCCA  GTCCACTTGT  AAGTCAAAAT  ATTCTTAATA
-----
12751  CAGATTCTAA  CTTGCTGTCA  GGCTGTAGAT  CTTCTTAAGC  TTGCGGGAGA
      GTCTAAGATT  GAACGACAGT  CCGACATCTA  GAAGAATTCT  AACGCCCTCT
-----

```

ApaI

```

12801  CGTCGAGTCC  AACCTGGGC  CCGATATCCC  CATGGTGAGC  AAGGGCGAGG
      GCAGCTCAGG  TTGGGACCCG  GGCTATAGGG  GTACCACTCG  TCCCGCTCC
-----
12851  AGCTGTTTAC  CGGGGTGGTG  CCCATCCTGG  TCGAGCTGGA  CGGCGACGTA
      TCGACAAGTG  GCCCCACCAC  GGGTAGGACC  AGCTCGACCT  GCCGCTGCAT
-----
12901  AACGGCCACA  AGTTCAGCGT  GTCCGGCGAG  GGCGAGGGCG  ATGCCACCTA
      TTGCCGGTGT  TCAAGTCGCA  CAGGCCGCTC  CCGCTCCCGC  TACGGTGGAT
-----
12951  CGGCAAGCTG  ACCCTGAAGT  TCATCTGCAC  CACCGGCAAG  CTGCCCGTGC
      GCCGTTTCGAC  TGGGACTTCA  AGTAGACGTG  GTGGCCGTTT  GACGGGCACG
-----
13001  CCTGGCCCCA  CCTCTGACC  ACCTTCACCT  ACGGCGTGCA  GTGCTTCAAG
      GGACCGGGTG  GGAGCACTGG  TGGAAGTGGA  TGCCGCACGT  CACGAAGTCG
-----
13051  CGCTACCCCG  ACCACATGAA  GCAGCACGAC  TTCTTCAAGT  CCGCATGCC
      GCGATGGGGC  TGGTGTACTT  CGTCGTGCTG  AAGAAGTTCA  GCGGTACGG
-----
13101  CGAAGGCTAC  GTCCAGGAGC  GCACCATCTT  CTTCAAGGAC  GACGGCAACT
      GCTTCCGATG  CAGGTCCTCG  CGTGGTAGAA  GAAGTTCCTG  CTGCCGTTGA
-----
13151  ACAAGACCCG  CGCCGAGGTG  AAGTTCGAGG  GCGACACCCT  GGTGAACCGC
      TGTTCGCGG  GCGGCTCCAC  TTCAAGCTCC  CGCTGTGGGA  CCACTTGGCG
-----
13201  ATCGAGCTGA  AGGGCATCGA  CTTCAAGGAG  GACGGCAACA  TCCTGGGGCA
      TAGCTCGACT  TCCCGTAGCT  GAAGTTCCTC  CTGCCGTTGT  AGGACCCCGT
-----
13251  CAAGCTGGAG  TACAACTACA  ACAGCCACAA  CGTCTATATC  ATGGCCGACA
      GTTCGACCTC  ATGTTGATGT  TGTCGGTGTT  GCAGATATAG  TACCGGCTGT
-----

```

13301 AGCAGAAGAA CGGCATCAAG GTGAACTTCA AGATCCGCCA CAACATCGAG
TCGTCTTCTT GCCGTAGTTC CACTTGAAGT TCTAGGCGGT GTTGTAGCTC

13351 GACGGCAGCG TGCAGCTCGC CGACCACTAC CAGCAGAACA CCCCCATCGG
CTGCCGTCGC ACGTCGAGCG GCTGGTGATG GTCGTCTTGT GGGGGTAGCC

13401 CGACGGCCCC GTGCTGCTGC CCGACAACCA CTACCTGAGC ACCCAGTCCG
GCTGCCGGGG CACGACGACG GGCTGTTGGT GATGGACTCG TGGGTCAGGC

13451 CCCTGAGCAA AGACCCCAAC GAGAAGCGCG ATCACATGGT CCTGCTGGAG
GGGACTCGTT TCTGGGGTTG CTCTTCGCGC TAGTGTACCA GGACGACCTC

13501 TTCGTGACCG CCGCCGGGAT CACTCTCGGC ATGGACGAGC TGTACAAGTA
AAGCACTGGC GGCGGCCCTA GTGAGAGCCG TACCTGCTCG ACATGTTTAT

Not I

13551 AAGCGGCCGC GACTCTAGGG GATTGCGJAT AAGTAAGTAA GCTTATGGAC
TTCGCCGGCG CTGAGATCCC CTAAGCGCTA TTCATTATT CGAATACCTG

13601 CTCAGAGAGG AAGTAACGAG GAGAGGGTGT GGTGGAATGT CACTAGAAAC
GAGTCTCTCC TTCATTGCTC CTCTCCCACA CCACCTTACA GTGATCTTTG

13651 CAGGGAAAAC AAGGAGGAGA GTATTACAGG GAAGGAGGTG AAGAACCTCA
GTCCCTTTTG TTCCTCCTCT CATATATGTC CTCTCTCCAC TTCTTGAGT

13701 TTACCCAAAT ACTCCTGCTC CTCATAGACG TACCTGGGAT GAGAGACACA
AATGGGTTTA TGAGGACGAG GAGTATCTGC ATGGACCCTA CTCTCTGTGT

13751 AGGTTCTTAA ATTGTCTCTCA TTCGCTACTC CCTCTGACAT CCAACGCTGG
TCCAAGAATT TAACAGGAGT AAGCGATGAG GGAGACTGTA GGTTGCGACC

13801 GCTACTAAAG CATTGCCTTA TGGCTGGAAA GTGGTCACCG AAAGCGGAAA
CGATGATTTC GTAACGGAAT ACCGACCTTT CACCAGTGGC TTTTCGCCCTT

13851 TGATTATACT AGCCGCAGAA AGATCAGAAC ATTGACAGAG ATGACTCAGG
ACTAATATGA TCGGCGTCTT TCTAGTCTTG TAAGTGTCTC TACTGAGTCC

13901 ATGAAATTAG AAAAAGGTGG GAAAGTGGAT ATTGTGACCC CTTTATTGAC
TACTTTAATC TTTTTCACAC CTTTACCTA TAACACTGGG GAAGTAAGTG

13951 TCAGGAAGTG ACTCAGATGG ACCCTTCTAA AAGCCACAGA CAGTAAAAAT
AGTCCTTCAC TGAGTCTACC TGGGAAGATT TTCGGTGTCT GTCATTTTTA

14001 GTGTTAGCAC TTTATACAAT ATTATATCTG CTTAAGCTAT AGAAGCTTTT
CACAAATCGTG AAATATGTGA TAATATAGAC GAATTCGATA TCTTCGAAAG

14051 ACATACTCAG TAGCTGTTTC ACAATCAACA AAACAATGAT GATGTAATCA
TGTATGAGTC ATCGACAAAG TGTTAGTTGT TTTGTTACTA CTACATTAGT

14101 TAAGGAAGTA GTTTAAATAG GTTAATAAGT TTATTAGTTA TATAGAAAAT
ATTCTTTCAT CAAATTTATC CAATTATTCA AATAATCAAT ATATCTTTTA

14151 AATATAGGAT AAAGTATAAG GATTAAGGTA TGAGGTGTGT GGCTCAACAC
TTATATCCTA TTTCATATTC CTAATTCCAT ACTCCACACA CCGAGTTGTG

14201 GTAGGGTGAC AAGAAAATCT ACTGTAATAG GACACAACAC CTCTAAAGTT
CATCCCACTG TTCTTTTAGA TGACATTATC CTGTGTTGTG GAGATTTCAA

SEQ ID NO: 2

a tgggtgctgag agcgtcggta ttaagcgggg gagaattaga
taaattgggaa
841 aaaattcggg taaggccagg gggaaagaaa caatataaac taaaacatat
agtatgggca
901 agcaggggagc tagaacgatt cgcagttaat cctggccttt tagagacatc
agaaggctgt
961 agacaaatac tgggacagct acaaccatcc cttcagacag gatcagaaga
acttagatca
1021 ttatataata caatagcagt cctctattgt gtgcatcaaa ggatagatgt
aaaagacacc
1081 aaggaagcct tagataagat agaggaagag caaaacaaaa gtaagaaaaa
ggcacagcaa
1141 gcagcagctg acacaggaaa caacagccag gtcagccaaa attaccctat
agtgcagaac
1201 ctccaggggc aatgggtaca tcaggccata tcacctagaa ctttaaattgc
atgggtaaaa
1261 gtagtagaag agaaggcttt cagcccagaa gtaataccca tgttttcagc
attatcagaa
1321 ggagccaccc cacaagattt aaataccatg ctaaaccacag tggggggaca
tcaagcagcc
1381 atgcaaatgt taaaagagac catcaatgag gaagctgcag aatgggatag
attgcatcca
1441 gtgcatgcag ggcctattgc accaggccag atgagagaac caaggggaag
tgacatagca
1501 ggaactacta gtacccttca ggaacaaata ggatggatga cacataatcc
acctatccca
1561 gtaggagaaa tctataaaaag atggataatc ctgggattaa ataaaatagt
aagaatgtat
1621 agccctacca gcattctgga cataagacaa ggaccaaagg aaccctttag
agactatgta
1681 gaccgattct ataaaactct aagagccgag caagcttcac aagaggtaaa
aaattggatg
1741 acagaaacct tgttgggtcca aaatgcgaac ccagattgta agactatttt
aaaagcattg
1801 ggaccaggag cgacactaga agaaatgatg acagcatgtc agggagtggg
gggacccggc
1861 cataaagcaa gagttttggc tgaagcaatg agccaagtaa caaatccagc
taccataatg
1921 atacagaaag gcaatttttag gaaccaaaga aagactgtta agtgtttcaa
ttgtggcaaa
1981 gaagggcaca tagccaaaaa ttgcagggcc cctaggaaaa agggctgttg
gaaatgtgga
2041 aaggaaggac accaaatgaa agattgtact gagagacagg ctaatttttt
agggaagatc
2101 tggccttccc acaaggggaag gccagggaat tttcttcaga gcagaccaga
gccaacagcc
2161 ccaccagaag agagcttcag gtttggggaa gagacaacaa ctccctctca
gaagcaggag
2221 ccgatagaca aggaactgta tccttttagct tccctcagat cactctttgg
cagcgacccc
2281 tcgtcacaat aa

SEQ ID NO: 3

MGARASVLSGGELDKWEKIRLRPGGKKQYKLKHIVWASRELERFAVNPGLLE
TSEGCRQILGQLQPSLQTGSEELRSYNTIAVLYCVHQRIDVKDTKEALDKIEEE

QNKSKKKAQQAAADTGNNSQVSQNYPIVQNLQGQMVHQAI SPRTLNAWVKV
VEEKAFSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDR LHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQNANPDCKTILKALGPGATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNPATIMI QKGNFRNQKRTVKCFNCGKEGHI AKNCRAPRKKGCWKC GKEGH
QMKDCTERQANFLGKIWPSHKGRPGNFLQSRPEPTAPPEESFRFGEETTPSQK
QEPIDKELYPLASLRS LFGSDPSSQ

SEQ ID NO: 4

MGARASVLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLE
TSEGC RQILGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEE
QNKSKKKAQQAAADTGHSSQVSQNYPIVQNIQGQMVHQAI SPRTLNAWVKV
VEEKAFSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNSATIMMQRGNFRNQKIVKCFNCGKEGHI ARNCRAPRKKGCWKC GKEG
HQMKDCTERQANFLGKIWPSYKGRPGNFLQSRPEPTAPPFLQSRPEPTAPPEES
FRSGVETTTPSQKQEPIDKELYPLTSLRS LFGNDPSSQ

SEQ ID NO: 5

MGARASVLSGGELDRWEKVRLRPGGKKKYKLKHIVWASRELERFAVNPGLLE
TSEGC RQILGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEE
QNKSKKKAQQAAADTGHSSQVSQNYPIVQNIQGQMVHQAI SPRTLNAWVKV
VEEKAFSPEVIPMFSA LSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAE
WDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKR
WIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMT
ETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQ
VTNSATIMMQRGNFRNQKIVKCFNCGKEGHI ARNCRAPRKKGCWKC GKEG
HQMKDCTERQANFLGKIWPSYKGRPGNFLQSRPEPTAPPFLQSRPEPTAPPEES
FRSGVETTTPSQKQEPIDKELYPLTSLRS LFGNDPSSQ

SEQ ID NO: 6

TCC GGG CCC GGA ATG CCT ATA GTC CAG AAC ATC C

SEQ ID NO: 7

GCG GCC GCG TTT TGA GAA CGA AAT ACC GG

SEQ ID NO: 8

SEQ ID NO:1 with SEQ ID NO: 2 inserted between 12816 and 13552.